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Research Article

The Effect of Different Phosphite Spray Formulas on Phytotoxicity and Phosphite Uptake of Avocado and Control Efficacy against *Phytophthora* Root Rot

Yu-Ping Liang[1] and Hui-Fang Ni[2,*]

Abstract

Liang, Y. P. and H. F. Ni. 2023. The effect of different phosphite spray formulas on phytotoxicity and phosphite uptake of avocado and control efficacy against *Phytophthora* root rot. J. Taiwan Agric. Res. 72(2):81–96.

Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* is one of the most important avocado diseases in Taiwan. Though foliar sprays of phosphonate fungicides have been used to manage this disease in other countries, the most effective application dosage and techniques have not been determined for avocado cultivars planted in Taiwan. To provide information for optimizing phosphite application strategies, this study investigated the effects of different phosphite spray formulas on avocado seedlings of various cultivars, focusing on phytotoxicity and phosphite uptake. The results showed that 0.1% and 0.2% phosphite caused mild (phytotoxicity score < 2) or no phytotoxic damage on seedlings of 6 avocado cultivars tested, including 'Choquette', 'Changan', 'Hung Shin Yuan', 'Zongpu Green Skin', 'Hall', and 'CAES3', while 0.5% phosphite could cause severe phytotoxic damage (phytotoxicity score ≥ 2) on 'Choquette', 'Changan', and 'Hall' under high temperature. In addition, pH had no obvious effect on phytotoxicity when the solutions were buffered to a pH ranging from 6.5 to 7.5. A detached root bioassay showed that, compared with the control, the colonization rates of *P. cinnamomi* were significantly lower in 'Changan' seedlings treated with phosphite and an adjuvant (0.2% phosphite plus Jia-Shou-Huo-Jhan (加收活展 , JSHJ), 0.5% phosphite plus JSHJ, or 0.5% phosphite plus S-408), and the root phosphite concentrations were above 180 μg g^{-1} for all these three treatments. Moreover, adding an adjuvant (JSHJ or S-408) to the phosphite solution reduced phytotoxic damage and increased the uptake of phosphite into the roots. The results generated from in this study will be helpful for the optimization of phosphite application strategies for the avocado industry.

Key words: Avocado, Adjuvant, Phosphite, *Phytophthora* root rot, Phytotoxicity.

INTRODUCTION

Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* is one of the most important avocado diseases not only in Taiwan but worldwide, resulting in tree decline, yield reduction, and even tree death. Methods currently used for management of PRR include the following: selection of good drainage sites; usage of resistant rootstocks, pathogen-free materials, and mulch; and implementation of proper irrigation practices, tree nutrition, and chemical controls (Dann *et al*. 2013).

Phosphonate fungicides (salts and esters of phosphite) have been shown to be effective

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in the management of avocado PRR (Pegg *et al*. 1985; Whiley *et al*. 2001; Ann *et al*. 2006; Dann *et al*. 2013). In plants, phosphonate fungicides ultimately dissociate into the relevant cations and phosphite anions (hydrogen phosphite [HPO_3^{-2}] and/or dihydrogen phosphite [$H_2PO_3^{-}$]), the latter of which are the active components involved in the suppression of *P.* spp. (Guest & Grant 1991; McDonald *et al*. 2001; Dann & McLeod 2021). The specific mode of action whereby phosphite suppresses avocado PRR is currently largely unknown, but it has been suggested that a sufficient level of phosphite in roots is required to protect the trees from serious PRR (Giblin *et al*. 2007; Dann *et al*. 2017). For example, in Australia, concentrations of 25–40 μg g^{-1} phosphite in avocado roots are considered necessary for root protection based on field observations (Giblin *et al*. 2007), while another study showed that at least 80 μg g^{-1} is required (Dann *et al*. 2017). The "sufficient level" of phosphite to suppress PRR might vary under different situations, as it could be related to the sensitivity of *P. cinnamomi* isolates to phosphite and the innate resistance level of rootstocks to PRR (Dann *et al*. 2017; Belisle *et al*. 2019). Therefore, the amount of phosphite required for effective control of PRR needs to be investigated for different cultivars and regions. Nevertheless, it has not been determined for avocado cultivars planted in Taiwan.

In avocado, phosphonate fungicides can be applied in various ways, including soil drench, trunk injection, foliar spray, and bark spray. There are a few peer-reviewed and non-peer-reviewed studies comparing the efficacy of different application methods. Soil drenches of phosphite had proved effective for PRR management in avocado seedlings (Ann *et al*. 2006). However, a study conducted with 10-year-old avocado trees in the field suggested that soil drenches only provided short-term (11 wk) protection (Pegg *et al*. 1985). Giblin *et al*. (2007) compared the effect of phosphonate trunk injections and bark sprays and found that root phosphite levels were generally higher in injected trees, although bark sprays also provided sufficient levels for controlling PRR. Trunk injections during the seasons when feeder roots are relatively strong sinks have been shown to have preventative and curative effects on avocado PRR (Pegg *et al*. 1985; Dann *et al*. 2013). Because of labor costs and possible trunk damage caused by injections, foliar spray application is considered an effective alternative (Dann *et al*. 2013; McLeod *et al*. 2018). In fact, for long-term and cost-effective control of PRR in Australia and South Africa, phosphonate fungicides are most often applied as foliar sprays or trunk injections (Dann *et al*. 2013; Masikane *et al*. 2020).

In Taiwan, to our best knowledge, only few growers apply phosphite to manage avocado PRR disease. Unlike that in other countries, phosphite is not applied annually during specific seasons, but applied as a soil drench when avocado trees are found to be in decline or as a foliar spray to apparent healthy trees at a rate of 0.1% phosphite before the rainy seasons to prevent PRR (unpublished data). Moreover, instead of using commercially available phosphonate products, most growers make phosphite solutions by dissolving equal weights of industrial grade phosphorous acid and potassium hydroxide into water right before application (Ann 2001). However, in a preliminary field trial, we found that spraying phosphite at a rate of 0.1% had limited effects on elevating the root phosphite concentration, and increasing application rate could lead to unacceptable phytotoxic damage, but the root phosphite concentration still did not reach a level sufficient for suppressing PRR (unpublished data).

Though the phytotoxic effects of phosphite and its accumulation in avocado have been investigated in Australia, South Africa, and the United States of America (USA) (Ouimette & Coffey 1989; Whiley *et al*. 2001; Dann *et al*. 2017; Masikane *et al*. 2020), the scion and rootstock cultivars used in Taiwan are different from those used in the above-mentioned countries. Instead of 'Hass', cultivars widely planted in Taiwan include 'Hall' (「厚兒」), 'Choquette' (「秋殼」), 'Changan' (「章安」), 'CAES3' (「嘉選 3 號」), and 'Hung Shin Yuan' (「紅心圓」) (Liang *et al*. 2021), which are also often used as rootstocks. Different avocado cultivars might respond

differently to phosphite, but the phytotoxic effects of phosphite and its accumulation in avocado roots have never been assessed on the cultivars planted in Taiwan.

The efficacy of phosphite uptake via foliar spray can be enhanced by applying appropriate adjuvants. Hardy *et al*. (2001) demonstrated that, upon control of *P. cinnamomi* by foliar application of phosphite in natural ecosystems, adding an adjuvant to phosphite increased spray coverage, promoted spray retention, as well as reduced spray drift, evaporation, and wash-off. Rolando *et al*. (2014) analyzed the effects of four commercial adjuvants and showed that the organosilicone-based adjuvant enhanced the uptake of phosphite into *Pinus radiata*. Though foliar application of phosphite has been used on avocado in many countries, there have been limited studies investigating the effects of different adjuvants on the uptake of phosphite in avocado.

The overall objective of this study was to provide useful information for optimizing phosphite application strategies to manage avocado PRR. The first aim was to evaluate the foliar phytotoxicity of phosphite on various avocado cultivars when applied at different dosages and with different pH values. The second aim was to assess the effects of adjuvants on phytotoxicity and the uptake efficacy of phosphite into avocado. The third aim was to evaluate the relationship between phosphite concentrations in avocado roots and the control efficacy against PRR by using a detached root bioassay. The information obtained in this study will help to optimize the phosphite application strategies for avocado.

MATERIALS AND METHODS

Preparation of phosphite solutions, cultivation of avocado seedlings, and evaluation of phytotoxicity

Phosphite solutions were prepared by dissolving crystal phosphorous acid (H_3PO_3, Grace Fertilizer Co., Ltd., Taichung, Taiwan) in reverse osmosis water. The pH value was adjusted to 7.2 by using potassium hydroxide (KOH, Grace Fertilizer Co., Ltd.) unless otherwise stated.

Avocado cultivars analyzed included ‘Choquette’, ‘Changan’, ‘Hung Shin Yuan’, ‘Zongpu Green Skin’ (「中埔青皮」), ‘Hall’, and ‘CAES3’. The seeds from each cultivars were grown in 4-inch pots in the greenhouse to generate the seedlings. To evaluate the phytotoxicity effects, avocado seedlings were sprayed until run off with 0%, 0.1%, 0.2%, or 0.5% phosphite solution, with each treatment involving five seedlings. After 2 wk, all leaves were cut from the plants and photographed together (Fig. 1). The total necrotic and unaffected areas of all leaves were then measured together by using Image J software (National Institute of Health, Bethesda, ML, USA) (Pride *et al*. 2020). Because the maximum total necrotic leaf area was about 25% of the total leaf area in this study, and the symptom looked severe when the necrotic leaf area was above 10%, the phytotoxicity score was rated on a scale of 0 to 4, where 0 = no necrosis, 1 = less than 2% necrotic leaf area, 2 = 25% necrotic leaf area, 3 = 5–10% necrotic leaf area, and 4 = more than 10% necrotic leaf area.

The experiment was conducted twice. In the first trial, the seedlings of ‘Changan’, ‘Hung Shin Yuan’, and ‘Zongpu Green Skin’ were 4–6 months old, while those of ‘Choquette’ were 13 months old. In the second trial, the seedlings of ‘Changan’, ‘Hung Shin Yuan’, ‘Zongpu Green Skin’, and ‘CAES3’ were 9–11 months old, while those of ‘Choquette’ and ‘Hall’ were 18 months old. The ambient temperatures in the greenhouse were recorded as 11–33℃ and 26–42℃ in the first and second trials, respectively. The ambient relative humidity was recorded as 39–94% in both trials.

Effect of pH on the phytotoxicity of phosphite sprays

To evaluate the effect of pH on the phytotoxicity of phosphite sprays, the seedlings of ‘Hall’, ‘Zongpu Green Skin’, and ‘CAES3’ were sprayed with 0.5% phosphite solution of different pH (6.5, 6.8, 7.2, or 7.5) and the phytotoxic ef-

Fig. 1. A representative photograph showing the pattern of phytotoxicity. The leaves were all from the same seedling, arranged from the oldest to the youngest in this photograph from top left to bottom right. This replicate seedling was 'CAES3' sprayed with 0.5% phosphite, in which the total necrotic area was calculated as 21%.

fects were evaluated as described in the previous section, with each treatment involving five replicate seedlings as well. The ambient temperature and relative humidity in the greenhouse were recorded as 18–40℃ and was 30–98%, respectively. The seedlings of 'Hall', 'Zongpu Green Skin', and 'CAES3' were about 16, 8, and 6 months old, respectively.

Inoculation of detached roots with *P. cinnamomi* zoospores and assessment of the colonization rate

The method for producing *P. cinnamomi* zoospores was adapted from Lonsdale *et al.* (1988). *P. cinnamomi* P69 was cultured on potato dextrose agar (PDA; Merck, Darmstadt, Germany) at 25℃ in darkness for 5 d. A Petri dish (9 cm in diameter) containing 2% V8 agar overlaid with moist miracloth (Merck, Darmstadt, Germany) was inoculated evenly with 10 pieces of agar blocks (2 × 2 mm) excised from the PDA culture. The plate was incubated in darkness for 7 d at 25℃, after which the miracloth was transferred to a 250 mL flask containing 100 mL of 2% V8 broth. The flask was shaken overnight in darkness at 25℃ at 160 rpm, and then

the miracloth was washed four times with 75 mL of mineral salt solution [0.01 M Ca $(NO_3)_2$, 0.005 M KNO_3, 0.004 M $MgSO_4$, and 1.3 mM Fe-EDTA] for 30 min at 160 rpm. After the final wash, 40 mL of the mineral salt solution was added to the flask, which was then shaken at 160 rpm overnight in darkness at 25℃. The miracloth was washed with 100 mL chilled (19℃) water, and then 10 mL of chilled water was added into the flask. The flask was incubated at 19℃ under light for 2–5 h to induce sporangium production and zoospore release. Zoospore concentration was quantified using a hemocytometer, and the concentration was adjusted to 1×10^4 zoospores mL^{-1} before use.

For the assessment of the colonization rate, three root tips (4 cm in length) were collected per seedling. After washing with tap water and rinsing with sterile water, the root tips were inoculated with *P. cinnamomi* P69 zoospores. The inoculation method was adapted from Van der Merwe & Kotzé (1994). Zoospore suspension (50 μL) was added to a 200 μL PCR tube. A detached avocado root tip was placed into the tube, with the root tip touching the bottom of the tube, and incubated in a moisture chamber at 25℃ for 2 h. Subsequently, the roots were transferred to Petri dishes with moist filter paper and incubated at 25℃ for 2 d. Colonization rates were determined by aseptically cutting the root tip into 10 segments (each ≈ 4 mm in length) after surface disinfection for 10 s in 70% ethanol. The root segments were then plated sequentially on a selective medium, PARPNB-PDA (PDA containing 10 mg L^{-1} pimaricin, 200 mg L^{-1} ampicillin, 8 mg L^{-1} rifampicin, 10 mg L^{-1} pentachloronitrobenzene, 50 mg L^{-1} nystatin, and 25 mg L^{-1} a.i. benomyl), adapted from Jung *et al.* (2000). After incubation at room temperature for 2 d, the number of segments from which *P. cinnamomi* grew was counted. The colonization rate was calculated using the following formula: colonization (%) = (the number of segments colonized/10) × 100%. The average percentage of three root tips from one seedling was shown as the value for one replicate.

Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *P. cinnamomi* colonization

'Hall', 'Choquette', and 'Changan' seedlings were sprayed until runoff with 0%, 0.2%, or 0.5% phosphite solution with 0.05% (v/v) adjuvant: Jia-Shou-Huo-Jhan (加收活展, JSHJ) (Sinon Co., Taichung, Taiwan) or S-408 (Microgreen, Ltd., Taichung, Taiwan). The labeled composition of JSHJ was "blend of polyethylene alkyl aryl ether and sodium salt of dialkyl sulfosuccinate" and that for S-408 was "10% silicone-isohexadecyl". Phosphite solutions without the adjuvants were also applied. There were five seedlings in each treatment. Two weeks after the treatment, the phytotoxic effects were assessed as aforementioned. Because growers are advised not to spray phosphite during leaf flush, phytotoxicity scores were also rated only on mature leaves.

In the trails using 'Choquette' and 'Changan', three root tips were collected per seedling for the assessment of *P. cinnamomi* colonization rate as aforementioned. The rest of the roots were sent to SGS Taiwan Ltd. for phosphite extraction and quantification. Because of technical limitations, at least 5 g of dry roots were required for processing each sample. Therefore, the roots from five replicates in each treatment were combined as one sample to be analyzed.

The ambient temperatures and relative humidity were recorded as 16–39℃/43–94%, 10–37℃/29–94%, and 15–40℃/27–92% in the trials of 'Hall', 'Choquette', and 'Changan', respectively. The seedlings of 'Hall', 'Choquette', and 'Changan' were about 15, 14, and 6 months old, respectively, in this experiment.

Quantification of phosphite in avocado roots

Roots obtained as described in the previous section were washed with tap water, blotted dried, and weighed to measure the fresh weights. Subsequently, they were placed in a brown paper bag, and dried at 50℃ in an oven for 5 d, followed by

measurement of the dry weights. Then, the dried roots were sent to SGS Taiwan Ltd. for phosphite extraction and quantification. The analytical method was adapted from McLeod *et al*. (2018) with the following modifications: (1) In the last step of phosphite extraction, the filtrate was centrifuged at 14,000 g for 40 min instead of 20 min. (2) For the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, the mobile phase consisted of solvent A (0.1% formic acid and 5 mM ammonium acetate in water) and solvent B (0.1% formic acid and 5 mM ammonium acetate in methanol). The LC gradient was as follows: 0 min, 99% A and 1% B; 2.5 min, 50% A and 50% B; 8.5 min, 30% A and 70% B; 12.5 min, 0% A and 100% B; 15.5 min, 0% A and 100% B; 15.6 min, 99% A and 1% B; and 18 min, 99% A and 1% B, with a flow rate of 0.3 mL min^{-1}. The column temperature was held at 50℃. The injection volume was 3 μL. The electrospray ionization source conditions in the negative ionization mode were as follows: a capillary voltage of 2.5 kV, source temperature of 150℃, and desolvation temperature of 400℃. The cone and desolvation gas flow rates were 150 and 800 L h^{-1}, respectively. Phosphite was detected using multiple reaction monitoring mode with the 80.9 > 63 transition at a collision energy of 20 eV. A recovery rate of 90% was used to adjust the LC/MS-MS value. Because most previous studies presented phosphite concentrations based on fresh weight (McLeod *et al*. 2018), the dry weight phosphite concentration was converted to fresh weight concentration according to the moisture content of each sample (85–90%). The total root phosphite quantity for each sample was then obtained by multiplying the root phosphite concentration by fresh weight.

Effect of adjuvants and pH on the phytotoxicity of phosphite sprays

‘Zongpu Green Skin’ and ‘CAES3’ were used for evaluating the effect of pH on the phytotoxicity when phosphite was applied with an adjuvant. Seedlings were sprayed with 0.5% phosphite plus 0.05% JSHJ solution at different pH values (6.5, 6.8, 7.2, and 7.5) until runoff. There were five replicate seedlings in each treatment. The ambient temperature and relative humidity in the greenhouse were recorded as 17–42℃ and 41–93%, respectively. The seedlings of ‘Zongpu Green Skin’ and ‘CAES3’ were about 8 and 6 months old, respectively, in this experiment.

Data analysis

The phytotoxicity scores were analyzed by using RStudio version 2022.02.3 (PBC, Boston, MA, USA). A Kruskal-Wallis test with a significance level of P = 0.05 was used to compare distributions of scores across treatments. If the Kruskal-Wallis test was significant, post-hoc analysis was performed using the Holm-corrected Dunn test to analyze differences between treatments.

The root colonization percentage data were transformed by arcsine transformation and subjected to one-way analysis of variance (ANOVA) followed by Fisher’s least significant difference (LSD) test using SAS Enterprise Guide version 7.15 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Phytotoxicity of phosphite sprays on different cultivars of avocado

Prior to phosphite application, all the avocado seedlings were healthy without any symptom of foliar necrosis. One day after the seedlings were sprayed with 0.2% or 0.5% phosphite, leaf discoloration, the initial symptom of phytotoxic effect, appeared on the margins of young leaves (Fig. 2A). Two days after application, the discolored area expanded and turned darker (Fig. 2B). Five days after application, the discolored area no longer expanded but dried up. Moreover, some young leaves became distorted due to the phytotoxicity of phosphite (Fig. 2C). Mild phytotoxic damage was observed as leaf tip burning and small necrotic spots on leaves (Fig. 2D), while large, dried areas developed in the case of more severe phytotoxic damage (Fig. 2E). Abscis-

sion of the dried spots was also observed in some cases (Fig. 2F). In addition, the degree of phytotoxicity might vary from leaf to leaf on the same seedling. Generally, younger leaves tended to be more susceptible to phosphite phytotoxicity and had higher percentages of necrotic area (Fig. 1).

In the first trial to analyze the phytotoxic effects of phosphite sprays, application of 0.1%, 0.2%, and 0.5% phosphite caused mild or no leaf burn on all cultivars (Table 1). However, the phytotoxic damage was more severe in the second trial. Phosphite applied at 0.5% caused mild leaf burn on ‘Hung Shin Yuan’, ‘Zongpu Green Skin’ and ‘CAES3’ (phytotoxicity score < 2), while it caused over 2% (score 2) necrotic leaf area on ‘Choquette’ and ‘Hall’, and over 5% (score 3) necrotic leaf area on ‘Changan’ (Table 1).

Effect of pH on the phytotoxicity of phosphite sprays

Assays to determine the phytotoxic effects of phosphite solutions with various pH values on avocado were conducted with 0.5% phosphite on ‘Hall’, ‘Zongpu Green Skin’, and ‘CAES3’. The phytotoxicity scores of ‘Hall’ and ‘Zongpu Green Skin’ were close to values obtained from assays of 0.5% phosphite without adjuvant in Table 1; the score for ‘Hall’ was about 2.4, while that for ‘Zongpu Green Skin’ was about 1–2. However, the average phytotoxicity score of ‘CAES3’ (3.2) was higher in this experiment compared to that shown in Table 1 (1.0 from Trial 2). There was no significant difference in the average phytotoxicity scores among different pH values for each cultivar as determined by the Kruskal-Wallis test (Table 2).

Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *P. cinnamomi* colonization

The average phytotoxicity scores were significantly lower for ‘Hall’ seedlings sprayed

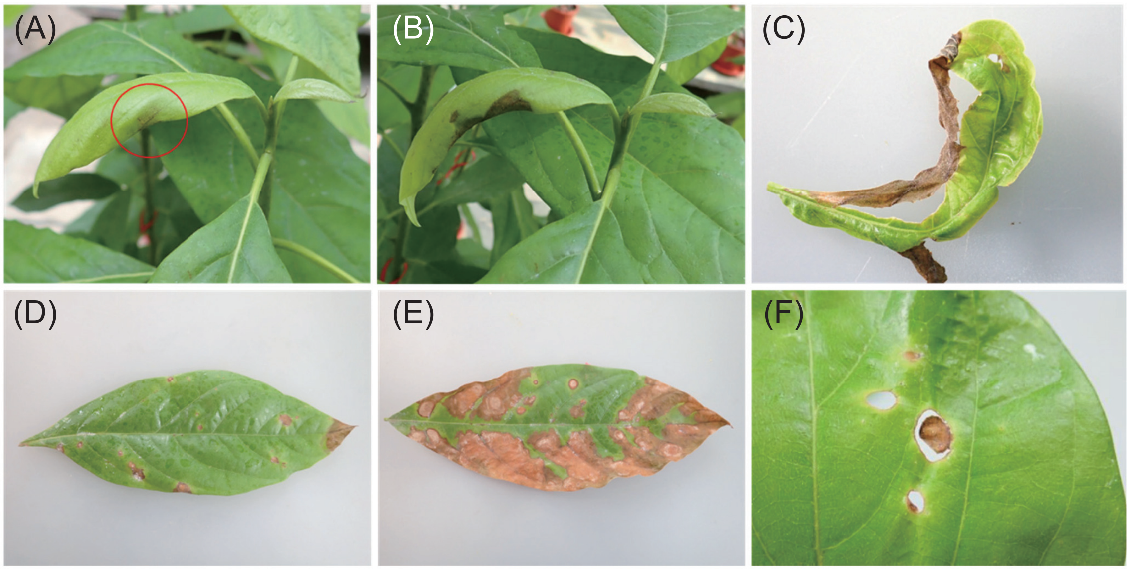

Fig. 2. Symptoms of phytotoxic damage caused by phosphite sprays on avocado leaves. (A) One day after application of a phosphite foliar spray, initial discoloration could be observed on the margins of new leaves of ‘Choquette’ sprayed with 0.2% phosphite. (B) Two days after application, the discolored area turned darker and the area enlarged. (C) New leaves of ‘Zongpu Green Skin’ sprayed with 0.5% phosphite distorted due to phytotoxicity. (D) Leaf tip burning and small necrotic spots observed on ‘Zongpu Green Skin’ leaves showing mild phytotoxicity damage from 0.5% phosphite. (E) Leaves of ‘CAES3’ with large necrotic areas, indicating severe phytotoxicity damage from 0.5% phosphite. (F) Abscission of the necrotic spots from ‘Choquette’ sprayed with 0.5% phosphite.

Table 1. Phytotoxic effects of phosphite sprays on various avocado cultivars.

	Phytotoxicity score (M ± SD)[z]					
Phosphite app. rate (%)	'Choquette'	'Changan'	'Hung Shin Yuan'	'Zongpu Green Skin'	'Hall'	'CAES3'
Trial 1						
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested
0.1	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	not tested	not tested
0.2	0.2 ± 0.4	0.4 ± 0.5	0.2 ± 0.4	0.0 ± 0.0	not tested	not tested
0.5	0.8 ± 0.4	1.0 ± 0.0	1.2 ± 0.4	1.0 ± 0.7	not tested	not tested
Trial 2						
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5
0.2	1.0 ± 0.0	1.0 ± 0.7	1.0 ± 0.7	1.0 ± 0.0	0.8 ± 0.4	0.8 ± 0.4
0.5	2.0 ± 1.2	3.0 ± 1.4	1.2 ± 1.4	1.8 ± 0.8	2.4 ± 1.3	1.0 ± 0.0

[z] Mean (M) and standard deviation (SD) were derived from five replicates.

Table 2. Phytotoxic effects of 0.5% phosphite sprays with various pH values on avocado.

	Phytotoxicity score (M ± SD)[zy]				
	Without adjuvant			With JSHJ[x]	
pH value	'Hall'	'Zongpu Green Skin'	'CAES3'	'Zongpu Green Skin'	'CAES3'
6.5	2.8 ± 1.3	1.2 ± 0.4	3.0 ± 1.4	1.0 ± 0.0	0.8 ± 0.4
6.8	2.8 ± 0.4	1.0 ± 0.7	3.2 ± 0.4	1.0 ± 0.0	1.0 ± 0.0
7.2	2.4 ± 0.9	1.0 ± 0.7	3.2 ± 0.8	0.8 ± 0.4	1.0 ± 0.0
7.5	3.8 ± 0.4	1.2 ± 0.4	2.4 ± 0.5	1.0 ± 0.0	1.0 ± 0.0

[z] Mean (M) and standard deviation (SD) were derived from five replicates.
[y] No significant difference among the average phytotoxicity scores of different pH values for each cultivar as determined by the Kruskal-Wallis test.
[x] JSHJ: Jia-Shou-Huo-Jhan (加收活展).

with 0.5% phosphite plus JSHJ or S-408 as compared with those sprayed with 0.5% phosphite without adjuvant (P = 0.036 and 0.014, respectively) (Table 3). The average phytotoxicity score for applying 0.2% phosphite plus S-408 was also significantly lower than that with 0.2% phosphite without adjuvant (P = 0.029) (Table 3). The adjuvants had less effect on phytotoxicity on 'Choquette' and 'Changan', which were less susceptible to phosphite phytotoxicity than 'Hall'. The addition of either JSHJ or S-408 adjuvant reduced phytotoxicity scores for both the 0.2% and 0.5% phosphite sprays, but only the 0.5% phosphite spray with JSHJ or S-408 on mature leaves of 'Choquette' significantly dropped the scores (Table 4). In contrast, analysis performed with 'Changan' indicated that only the 0.2% phosphite spray plus S-408 significantly lowered the scores (Table 5). In some plants, phytotoxic damage was observed only on immature leaves. In 'Choquette', no phytotoxic damage was observed on mature leaves of seedlings treated with phosphite plus adjuvant sprays. Application of JSHJ or S-408 alone caused no phytotoxic damage.

In control plants without phosphite spray application, the root phosphite concentrations were very low, with 1.6 and 1.7 μg g^{-1} for 'Choquette' (Table 4) and 'Changan' (Table 5), respectively. In contrast, the root phosphite concentrations and quantities were all elevated by over

Table 3. Effect of adjuvants on the phytotoxicity on 'Hall' seedlings.

Phosphite app. rate (%)	Adjuvant	Phytotoxicity score (M ± SD)[z]
0.0	-	0.0 ± 0.0
0.2	-	0.8 ± 0.4 a
0.2	JSHJ[y]	0.2 ± 0.4 ab
0.2	S-408	0.0 ± 0.0 b
0.5	-	2.2 ± 0.8 a
0.5	JSHJ	1.0 ± 0.0 b
0.5	S-408	0.8 ± 0.4 b

[z] Mean (M) and standard deviation (SD) were derived from five replicates. Values with the same phosphite application rate followed by different letters are significantly different according to Holm-corrected Dunn test ($P < 0.05$).

[y] JSHJ: Jia-Shou-Huo-Jhan (加收活展).

Table 4. Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *Phytophthora cinnamomi* colonization on 'Choquette' seedlings.

Phosphite app. rate (%)	Adjuvant	Phytotoxicity score (M ± SD)[z]	Phytotoxicity score on mature leaves (M ± SD)[z]	Root phosphite conc. (μg g^{-1})	Total root phosphite quantity (mg)[y]	Root colonization (M ± SD, %)[x]
0.0	-	0.0 ± 0.0	0.0 ± 0.0	1.6	0.1	60.0 ± 15.5 a
0.0	JSHJ[w]	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.0	S-408	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.2	-	0.4 ± 0.5 a	0.4 ± 0.5 a	18.7	1.4	46.7 ± 20.5 ab
0.2	JSHJ	0.2 ± 0.4 a	0.0 ± 0.0 a	78.7	7.3	44.0 ± 23.5 ab
0.2	S-408	0.0 ± 0.0 a	0.0 ± 0.0 a	18.8	2.2	41.3 ± 8.0 ab
0.5	-	1.0 ± 0.7 a	1.0 ± 0.7 a	17.2	1.8	36.0 ± 14.4 b
0.5	JSHJ	0.4 ± 0.5 a	0.0 ± 0.0 b	111.8	10.9	35.3 ± 9.6 b
0.5	S-408	0.4 ± 0.5 a	0.0 ± 0.0 b	78.7	4.8	28.0 ± 11.7 b

[z] Mean (M) and standard deviation (SD) were derived from five replicates. Values with the same phosphite application rate followed by different letters are significantly different according to Holm-corrected Dunn test ($P < 0.05$).

[y] The total quantity of phosphite from five replicate seedlings in each treatment.

[x] M and SD were derived from five replicates. Values followed by different letters are significantly different according to Fisher's least significant difference test ($P < 0.05$).

[w] JSHJ: Jia-Shou-Huo-Jhan (加收活展).

10 folds after treatment with phosphite spray. In addition, the quantities of phosphite were all higher in 'Choquette' (Table 4) and 'Changan' (Table 5) seedlings treated with phosphite plus adjuvants compared to treatment with phosphite at the same rate without adjuvants.

Generally, the addition of JSHJ yielded higher root phosphite quantities than S-408 under the same phosphite application rate, and the treatments with 0.5% phosphite yielded higher root phosphite quantities than those with 0.2% phosphite with the same adjuvant. The only one exception was 'Changan' seedlings sprayed with 0.5% phosphite plus JSHJ, in which the root phosphite quantity (6.9 mg) was lower than that of seedlings sprayed with 0.2% phosphite plus JSHJ (9.2 mg) or 0.5% phosphite plus S-408 (8.5 mg). In 'Choquette', treatment with 0.5% phosphite plus JSHJ yielded the highest root phosphite concentration (111.8 μg g^{-1}) and quantity (10.9 mg), while in 'Changan', treatment with 0.2% phosphite plus JSHJ yielded the highest root phosphite quantity (9.2 mg), and treatment with

Table 5. Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *Phytophthora cinnamomi* colonization on 'Changan' seedlings.

Phosphite app. rate (%)	Adjuvant	Phytotoxicity score (M ± SD)[z]	Phytotoxicity score on mature leaves (M ± SD)[z]	Root phosphite conc. (μg g^{-1})	Total root phosphite quantity (mg)[y]	Root colonization (M ± SD, %)[x]
0.0	-	0.0 ± 0.0	0.0 ± 0.0	1.7	0.1	62.7 ± 27.5 a
0.0	JSHJ	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.0	S-408	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.2	-	0.8 ± 0.4 a	0.6 ± 0.5 a	35.2	1.6	39.3 ± 23.3 abc
0.2	JSHJ	0.4 ± 0.5 ab	0.2 ± 0.4 a	183.1	9.2	18.7 ± 11.9 cd
0.2	S-408	0.0 ± 0.0 b	0.0 ± 0.0 a	104.0	5.8	40.0 ± 13.9 abc
0.5	-	1.2 ± 0.4 a	1.2 ± 0.4 a	108.5	5.7	42.0 ± 20.6 ab
0.5	JSHJ	1.0 ± 0.0 a	1.0 ± 0.0 a	199.1	6.9	13.3 ± 8.5 d
0.5	S-408	1.0 ± 0.0 a	1.0 ± 0.0 a	190.8	8.5	29.3 ± 15.9 bcd

[z] Mean (M) and standard deviation (SD) were derived from five replicates. Values with the same phosphite application rate followed by different letters are significantly different according to Holm-corrected Dunn test ($P < 0.05$).

[y] The total quantity of phosphite from five seedlings in each treatment.

[x] M and SD were derived from five replicates. Values followed by different letters are significantly different according to Fisher's least significant difference test ($P < 0.05$).

0.5% phosphite plus JSHJ yielded the highest root phosphite concentration (199.1 μg g^{-1}) (Tables 4 and 5).

The root colonization rates were significantly lower in the 'Choquette' seedlings sprayed with 0.5% phosphite alone, 0.5% phosphite plus JSHJ, or 0.5% phosphite plus S-408 as compared with the control, and the root phosphite concentrations ranged from 17.2 to 111.8 μg g^{-1} for these treatments. The three treatments with 0.2% phosphite resulted in a similar range of root phosphite concentrations (18.7–78.7 μg g^{-1}), but the root colonization rates were not significantly lower than that of the control (Table 4). In the 'Changan' seedlings sprayed with 0.2% phosphite plus JSHJ, 0.5% phosphite plus JSHJ, or 0.5% phosphite plus S-408, the average root colonization rates were all lower than 30%, which was significantly different from that in the control (62.7%), and the root phosphite concentrations were all above 180 μg g^{-1} (Table 5).

Effects of adjuvants and pH on the phytotoxicity of phosphite sprays

When 0.5% phosphite was applied along with the adjuvant JSHJ, the average phytotoxicity scores were 1.0 or 0.8 for all pH values for both 'Zongpu Green Skin' and 'CAES3' seedlings, and there were no significant differences in the average phytotoxicity scores among the treatments with pH values ranging from 6.5 to 7.5, as determined by the Kruskal-Wallis test (Table 2).

DISCUSSION

Instead of being uniformly planted with the 'Hass' cultivar, most avocado orchards in Taiwan are planted with various cultivars. Therefore, at the beginning of this study, various cultivars were sprayed with phosphite solutions at different rates to evaluate the phytotoxic effects of phosphite on them. The results suggested that susceptibility to phosphite might vary among different cultivars. As there are diverse cultivars planted in Taiwan, more than what were included in this study, the susceptibility of more avocado cultivars to phosphite needs to be investigated in the future. Besides, genetically identical grafted plants should be used for the assessment of susceptibility to provide more reliable results. Before then, growers should test the application rate on a small scale before

applying it to the whole orchard. In addition, the phytotoxic damage was more severe in the second trial, in which the ambient temperature in the greenhouse was higher than that in the first trial. The results suggested that the occurrence of phytotoxicity would increase when phosphite was applied under high temperature (above 35℃). Therefore, growers should be more careful when applying foliar phosphite sprays under high temperature.

An inconsistent result in this study was that the average phytotoxicity score of 'CAES3' sprayed with 0.5% phosphite was only 1.0 as shown in Table 1, but it was 3.2 in Table 2. One possible explanation for this discrepancy is that the 'CAES3' seedlings were older in the former experiment (10 months old) than in the latter (6 months old). The effects of plant age on phytotoxicity should be investigated in future studies to test this hypothesis.

It is suggested that the most effective times to apply phosphite are the stages when the spring and summer flush leaves have matured because feeder roots are acting relatively as stronger sinks at these two stages (Dann *et al*. 2013). In this study, we also found that phosphite could cause deformation of immature leaves. The findings further support the advice that growers should avoid applying phosphite when there are immature leaves on the trees.

In Taiwan, most growers make phosphite solutions by dissolving industry grade H_3PO_3 and KOH at equal weights into water right before application. However, making the solution with different sources of water might result in different pH values. For example, the pH values of phosphite solutions made by dissolving equal weights of H_3PO_3 and KOH into reverse osmosis water (pH 6.8), underground water (pH 7.4), and a different source of underground water (pH 7.9) were 6.45, 6.5, and 7.5, respectively (unpublished data). To provide guidelines for growers for preparation of phosphite solutions, whether it is necessary to adjust the pH value to a certain range was investigated in this study. Whiley *et al*. (2001) compared the phytotoxicity of foliar-applied phosphite solutions with different pH values ranging from 6.8 to 7.6 and showed that the least phytotoxic damage on 'Hass' leaves was observed at pH 7.2. Therefore, it was suggested that foliar-applied phosphite solutions be buffered to pH 7.2 before application (Dann *et al*. 2013). However, we found no significant difference among phosphite treatments with different pH values ranging from 6.5 to 7.5. One possible explanation for this discrepancy is that the sensitivity of the cultivars used in this study to phosphite solutions with various pH values was different from that of 'Hass'. Another possible explanation is that the sensitivity of seedlings in the greenhouse to phosphite was different from that of mature trees in the field. Further studies should be conducted in the field with 'Hass' and grafted mature trees to test these hypotheses.

During the experiments, we observed that phosphite sprays formed droplets on the leaves due to their slight hydrophobicity, and the droplets left sticky patches when they dried up. Therefore, we speculate that the uneven distribution of phosphite on leaves might increase the risk of phytotoxicity and that prompted us to investigate the possibility of adding adjuvants to reduce the phytotoxic damage.

Though the effect of adding adjuvants into foliar phosphite sprays has been tested in a few studies (Hardy *et al*. 2001; Rolando *et al*. 2014), very few studies focused on the effects of adjuvants on avocado. Ouimette & Coffey (1989) added 0.1% Triton B-1956 as an adjuvant to a foliar spray of potassium phosphonate, but there was no comparison of the phosphite uptake between the treatments with or without the adjuvant. A non-peer-reviewed study showed that applying phosphite with the adjuvant Agral® or Nufilm® significantly increased the risk of phytotoxicity (Whiley *et al*. 2001). To our best knowledge, the present study is the first one investigating the effect of an adjuvant on phytotoxicity and uptake of phosphite on avocado simultaneously. Our

study showed that adding adjuvants not only decreased the risk of phytotoxicity, but also increased the uptake of phosphite into roots. In the experiments with 'Choquette' and 'Changan', spraying 0.2% phosphite containing adjuvants even resulted in a higher total root phosphite quantity than spraying 0.5% phosphite without adjuvant, which means less chemical would be needed if spraying with adjuvants and the cost of application could be reduced. However, it is possible that different adjuvants might have different effects on phytotoxicity when applied with phosphite, but only two adjuvants were tested in this study. The effect of more other adjuvants warrants further investigation. As there is a wide range of active ingredients in adjuvants, and some of them, such as Agral® or Nufilm®, might increase the risk of phytotoxicity (Whiley *et al.* 2001), growers should test combinations on a small scale before applying them to the whole orchard.

The root phosphite concentrations were all relatively lower in 'Choquette' than those in 'Changan' seedlings treated with phosphite. It is possible that different cultivars might have different abilities to accumulate phosphite in roots, which was suggested by Dann *et al.* (2017). This variation could also possibly result from the differences in root mass between the two cultivars. The root fresh weight per treatment (five seedlings) for 'Choquette' was 60–120 g but was 30–60 g for 'Changan' (data not shown). As 'Choquette' seedlings had greater root mass than 'Changan', the absorbed phosphite might have been diluted to a lower concentration. This could also explain why spraying 'Choquette' with 0.2% phosphite alone yielded the same phosphite concentration as that with 0.2% phosphite plus S-408: the seedlings in the latter treatment happened to have a greater root mass. This observation might imply that the ratio of roots to leaves could influence the final concentration of root phosphite.

Generally, the treatments with JSHJ yielded a higher total root phosphite quantity than with S-408 under the same phosphite application rate, which means JSHJ might be a better adjuvant than S-408 when only their ability to enhance uptake efficiency is considered. The finding that spraying 'Changan' with 0.5% phosphite plus JSHJ did not result in a higher root phosphite quantity than spraying with 0.2% phosphite plus JSHJ or 0.5% phosphite plus S-408 is confusing. One possible explanation is that seedlings in this treatment happened to have less leaf area for absorbing phosphite. Despite the inconsistency, phosphite uptake was consistently enhanced by adding adjuvants in this study.

In the detached root bioassay, the root phosphite concentrations of 'Changan' seedlings were all above 180 μg g^{-1} for the 0.2% phosphite plus JSHJ, and 0.5% phosphite plus JSHJ or S-408 treatments, in which the root colonization rates were all significantly lower compared with those of the control. This result might suggest that a high concentration of phosphite in roots is necessary for controlling PRR. However, the "sufficient" level of root phosphite concentration still could not be determined, because the colonization rates between the 0.2% phosphite and 0.5% phosphite plus S-408 treatments were not significantly different, even though there was a difference of 155.6 μg g^{-1} in the root phosphite concentration between them.

The relationship between the root phosphite concentration and colonization rate in the experiment with 'Choquette' seedlings was also inconsistent, as treatments resulting in the same root phosphite levels had significantly lower colonization rates than the control in some cases but not in others. A possible explanation is that none of the root phosphite levels in 'Choquette' seedlings treated with phosphite sprays reached a level sufficient to make a difference in control efficacy. In addition, because of technical and financial limitations (approximately 195 USD per sample), the roots from each treatment were mixed together for phosphite quantification, instead of each replicate seedling being analyzed separately.

The variation of root phosphite concentration among replicate seedlings could be high because the root mass of each seedling was different, and this might result in the inconsistency between the root phosphite concentration and colonization rate. It is also possible that other mechanisms such as induced resistance might be involved. More research needs to be conducted to clarify the relationship between root phosphite concentration and colonization rate and to investigate the specific mechanisms relating to how phosphite inhibits colonization and that will be useful for managing avocado PRR.

Phosphite is frequently used as an environmentally friendly protectant for controlling various plant diseases in Taiwan, but no grower quantifies phosphite concentration in plant tissues before or after application, mostly because there is no facility providing phosphite quantification service in Taiwan, and phosphite is thought to control diseases by inducing resistance at low concentrations (Ann 2001). We propose that quantification of phosphite in avocado is important for the following reasons. Firstly, there is evidence that a sufficient level of phosphite in roots is required to suppress avocado PRR (Giblin *et al*. 2007; Dann *et al*. 2017). In fact, the current strategy of phosphonate application in Australia involves quantifying phosphite concentration in feeder roots before and after application for growers to determine whether they should apply or re-apply phosphonate fungicides, which might be a good model for phosphite application in Taiwan. Secondly, although potassium phosphonates are considered harmless to human health, some countries still set a maximum residue limit (MRL) for potassium phosphonate products in avocado. For example, the European Food Safety Authority (EFSA) considers an acceptable daily intake (ADI) of phosphonic acid to be 1 mg kg^{-1} applicable, and the MRL of phosphonic acid in avocado was set at 50 mg kg^{-1} (European Food Safety Authority *et al*. 2020). Though in Taiwan there is no MRL set for avocado in the domestic market and no avocado is currently exported, accurate phosphite quantification techniques might become necessary in the future, especially if the industry intends to pursue foreign markets.

Several analytical methods have been published for quantifying phosphite in plant tissues, including high performance ion chromatography, LC-MS/MS, and gas chromatography–mass spectrometry (Roos *et al*. 1999; Barrett *et al*. 2003; Berkowitz *et al*. 2011; McLeod *et al*. 2018). The advantages and disadvantages of these methods have been reviewed in McLeod *et al*. (2018). LC-MS/MS was chosen as the analytical method in this study because it is used by commercial laboratories for quantifying phosphite in avocado roots in Australia and New Zealand. This study adapted and introduced the analytical method for phosphite to a commercial laboratory (SGS Taiwan Ltd.) in Taiwan. We hope that sending roots for phosphite quantification will become a common practice for avocado growers to optimize the application of phosphite in the future.

In conclusion, this study suggested that: (1) the susceptibility to phosphite phytotoxicity might vary among different avocado cultivars; (2) the pH value of a phosphite spray might have limited effect on phytotoxicity when the solution is buffered to a pH between 6.5 and 7.5; and (3) adding adjuvants might reduce the risk of phytotoxicity and increase the uptake of phosphite. Since the experiments were all conducted with non-grafted seedlings in the greenhouse, they might not represent exactly the effects on grafted mature trees in the field. Therefore, future studies with mature trees are warranted. The analytical method used for phosphite quantification and the results derived from this study could provide important information for optimization of phosphite application strategies and future studies on managing avocado PRR.

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不同亞磷酸配方對酪梨之藥害程度、亞磷酸吸收及根腐病防治效果之影響

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摘要

梁鈺平、倪蕙芳。2023。不同亞磷酸配方對酪梨之藥害程度、亞磷酸吸收及根腐病防治效果之影響。台灣農業研究 72(2):81–96。

由 *Phytophthora cinnamomi* 造成之根腐病 (*Phytophthora* root rot) 為台灣酪梨最重要之病害。雖然在國外，以葉面噴施亞磷酸防治酪梨根腐病由來已久，但針對國內酪梨品種，至今仍缺乏有效施用亞磷酸劑量及技術之研究。為優化亞磷酸防治酪梨根腐病施用策略所需資訊，本研究以國內常見品種之酪梨苗測試不同亞磷酸配方對酪梨葉片藥害及亞磷酸吸收之影響。實驗結果顯示，於測試的 6 個品種 (「秋殼」、「章安」、「紅心圓」、「中埔青皮」、「厚兒」及「嘉選三號」) 上，葉面噴施 0.1% 及 0.2% 之亞磷酸於酪梨苗造成之藥害均極輕微 (藥害級數 < 2)，而 0.5% 亞磷酸則可能於高溫下對「秋殼」、「章安」及「厚兒」造成嚴重藥害 (藥害級數 ≥ 2)。若亞磷酸已中和至 pH 值介於 6.5–7.5 之間，則 pH 值對藥害程度影響無顯著差異。於「章安」之離根接種實驗結果顯示，噴施 0.2% 亞磷酸 + 加收活展、0.5% 亞磷酸 + 加收活展，或 0.5% 亞磷酸 + S-408 處理之根系 *P. cinnamomi* 感染率均顯著低於對照組，且此些處理酪梨根系之亞磷酸濃度均大於 180 $\mu g\ g^{-1}$。此外，於亞磷酸溶液中添加展著劑 (加收活展或 S-408) 可減少藥害並增加根部對亞磷酸之吸收。本研究結果有助於優化亞磷酸在酪梨產業的應用策略。

關鍵詞：酪梨、展著劑、亞磷酸、根腐病、藥害。

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研究報告

雙重酶切 DNA 定序技術應用於番椒種原基因體分析

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摘要

林思妤、王昭月、陳涵葳、李承彬、林大鈞、杜元凱。2023。雙重酶切 DNA 定序技術應用於番椒種原基因體分析。台灣農業研究 72(2):97–112。

次世代定序技術已成為作物基因體研究相當常見的工具，而為集中定序資源與降低定序成本，已逐漸發展出多種簡化基因體定序技術，然而不同作物、不同族群組成所適宜的文庫建置方法 (如限制酶選擇或是定序量等) 有差異，若能針對目標作物及族群組成進行方法上的調整，將可使定序資源發揮最大效率。本研究使用簡化基因體技術之一：雙重酶切 DNA 定序技術 (double-digest restriction site-associated DNA sequencing; ddRAD-seq) 針對不同種番椒 (*Capsicum* spp.) 種原基因體進行分析，第一部分比較 *Pst*I/*Msp*I 及 *Pst*I/*Mse*I 限制酶酵素組合，電腦模擬結果顯示兩種組合產生的可被定序片段為總片段之 2.01% 及 0.34%，而實際 2 個酵素組合的定序結果顯示，以 *Pst*I/*Msp*I 組合 (19–28 M) 比 *Pst*I/*Mse*I 組合 (15–17 M) 得到較多成對且比對專一的讀序，因此不論在建庫資源利用上或是成功對應回參考序列的讀序數量/比例上，*Pst*I/*Msp*I 皆較 *Pst*I/*Mse*I 表現佳。本研究第二部分針對 28 個番椒收集系 (涵蓋 5 個番椒馴化種) 以 *Pst*I/*Msp*I 酶切並進行 ddRAD 定序，共得到 14,230 個高品質單一核苷酸多型性 (single nucleotide polymorphism; SNP)，平均分布於番椒 12 對染色體並以染色體兩端基因較密集處較多；利用此些 SNP 進行集群分析，亦能成功將不同種番椒品系正確分類。此說明本研究使用的一系列建庫、定序、生物資訊分析等方法，可用於橫跨不同種的番椒基因體分析，未來將以此為基礎，針對台灣番椒種原建立其基因體資訊，作為台灣番椒育種的根基。

關鍵詞：簡化基因體定序、雙重限制酶、番椒、基因體分析。

前言

番椒 (*Capsicum* spp.) 包含辣椒及甜椒，為全球第二大的茄科作物，具極高經濟價值。其最早起源自中南美洲，為人類利用已有 5,000–6,000 年歷史，目前甜椒主要產區為歐洲及美洲的溫帶及亞熱帶地區，辣椒產區則遍布各大洲熱帶及亞熱帶地區。番椒屬目前已發現至少涵蓋 38 個種，其中 *Capsicum annuum*、*C. baccatum*、*C. chinense*、*C. frutescens* 及 *C. pubescens* 為馴化種，當中又以 *C. annuum* 最為廣泛栽培及應用於育種，且由於長期的人為選拔，在果實大小、形狀、顏色及辣度上皆有非常高的多樣性 (Du *et al*. 2019)。然而面對氣候變遷及人們不斷地求新求變，有必要持續進行番椒品種改良以兼顧栽培及市場偏好。種原為作物改良的基礎，若能瞭解番椒種原的基因體資訊，將有助於加速新品種育成。

目前番椒已有數個全基因體定序資料釋出，Kim *et al*. (2014) 釋出第一個番椒全基因體定序 *C. annuum* ‘CM334’，同年 Qin *et al*. (2014) 釋出 *C. annuum* ‘Zunla-1’ 及 ‘Chiltepin’ (*C. annuum* var. *glabriusculum*) 的定序資料，而後 Kim *et al*. (2017) 針對 *C. chinense* ‘PI159236’ 及 *C. baccatum* ‘PBC81’ 進行解序，Hulse-Kemp *et al*. (2018) 完成 *C. annuum* 一代雜交種 (F_1) 的全基因體定序。此些研究皆顯示番椒基因

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體相當大 (約 3−3.5 Gb) 且基因體十分複雜，全基因體 80% 以上由重複序列 (repetitive element) 組成，約具有 35,000 個基因。

全基因體定序所需費用相當高昂，因此西元 2000 年後逐漸發展出簡化基因體定序相關技術，如 reduced representation shotgun sequencing (RRS) (Altshuler *et al*. 2000)、genotyping-by-sequencing (GBS) (Elshire *et al*. 2011) 及 restriction site-associated DNA sequencing (RADseq) (Baird *et al*. 2008) 等技術，上述技術皆是利用定序特定限制酶剪切位置兩側的核酸序列，亦即依據所使用的限制酶，僅定序大約 0.5–4% 的全基因體組核酸序列，因此可大幅降低每個樣本所需定序費用。基於簡化基因體定序概念，後續衍伸出相當多相似的方法，截至 2017 年已至少有 36 篇方法學的發表，現今亦已廣泛應用於多種作物之基因定位、族群結構分析及親緣分析等研究 (Campbell *et al*. 2018)。

目前已有多篇研究利用簡化基因體定序技術進行番椒種原相關研究，如 Han *et al*. (2018) 以 GBS 定序技術進行 208 個番椒品系 (*C. annuum*、*C. chinense* 及 *C. frutescens*) 辣椒素基因座探勘，Ramchiary & Kole (2019) 利用 double-digest RAD (ddRAD) 分析 5 個番椒馴化種 (186 個品系) 之果實方向性，Pereira-Dias *et al*. (2019) 以 GBS 分析 190 個番椒品系 (橫跨至少 5 個種) 之遺傳歧異度及族群結構，Potnis *et al*. (2019) 則以 GBS 為基礎進行 287 個美國農部番椒種原抗細菌性斑點病 (*Xanthomonas gardneri*) 基因座定位。進行簡化基因體定序時，限制酶的選擇相當重要，會影響到酶切後片段數目及大小，從而影響到讀序 (reads) 及單一核苷酸多型性 (single nucleotide polymorphism; SNP) 的數量及分布。綜上各篇利用簡化基因體定序技術於番椒種原相關研究文獻，目前常見以 1 個限制酶 (*Ape*KI) 酶切 (Nimmakayala *et al*. 2016; Taitano *et al*. 2019)，亦有用 2 個限制酶酶切者，如 *Pst*I/*Msp*I (Yamazaki *et al*. 2020) 或 *Pst*I/*Mse*I 組合 (Han *et al*. 2018)。

本研究團隊將針對台灣種原庫中的 4,800 多份番椒種原進行基因體研究，並以基因型資料建立核心種原，由於番椒種原數量龐大，且基因體亦相當龐大 (3–3.5 Gb)，因此有必要進行先期試驗，以找到定序資源利用效率最佳的方法，並初步對番椒種原進行遺傳背景分析。本研究分為 2 部分，第一部分以電腦模擬 (*in silico*) 及活體外試驗 (*in vitro*) 評估 2 種番椒常見的限制酶酵素組合—*Pst*I/*Msp*I 及 *Pst*I/*Mse*I 用於 ddRAD 中的效率，第二部分則對 28 個番椒收集系進行遺傳歧異度的分析，以對番椒種原之遺傳背景有初步瞭解。

材料與方法

植物材料及 DNA 萃取

本研究番椒品系來自行政院農業委員會農業試驗所之國家作物種原中心種原庫及育種材料，涵蓋 5 個番椒馴化種，包含 17 個 *C. annuum*、3 個 *C. baccatum*、3 個 *C. chinense*、2 個 *C. frutescens*、2 個 *C. pubescens* 及 1 個未知種 *C.* sp.，合計共 28 個收集系 (表 1)。28 個收集系中有 10 個收集系屬自交系 (名稱為 SP 開頭)，經自交純化 4–6 代，其他 18 個收集系則來自種原庫，未經自交純化。此些番椒品系於 2020 年 10 月栽培於農業試驗所溫網室 (位於台中霧峰)，採離土栽培，肥培管理依照慣行農法進行。

每個品系採摘同一單株 2 片嫩葉冷凍乾燥備用，再以 DNeasy Plant Mini kit (Qiagen GmbH, Hilden, Germany) 進行 DNA 萃取，最後以 Qubit dsDNA BR 試劑及 Qubit 螢光儀 (Thermo Fisher Scientific, Waltham, MA, USA) 進行 DNA 定量，並以 1% agarose gel 確認 DNA 品質。

雙重限制酶組合評估

ddRAD-seq 概念為使用 1 稀疏切位 (rare cutter) 以及 1 常用切位 (common cutter) 之限制酶剪切作物基因體核酸，以獲取合適數目與大小基因體核酸片段進行定序，進而達到簡化基因組定序效果 (Elshire *et al*. 2011)。考量試驗進行成本 (限制酶用量、建庫及定序費用)，又相關研究指出使用甲基化敏感之限制酶較易於染色體富含基因區域進行剪切 (Han *et al*. 2018)，綜合上述因素以及常用在番椒 ddRAD-

表 1. 參試番椒樣本清單。

Table 1. Sample list of *Capsicum* spp.

ID	Species	Name	Heterozygous proportion
P0133	*C. annuum*	01A00493	0.079
P0134	*C. annuum*	01A00494	0.101
P0164	*C. annuum*	05A01225	0.106
P0287	*C. annuum*	97A05512	0.084
P0565	*C. annuum* var. *aviculare*	95A04169	0.116
P1025	*C. annuum*	SP20	0.128
P1027	*C. annuum*	SP40	0.110
P1028	*C. annuum*	SP41	0.071
P1032	*C. annuum*	SP63	0.110
P1033	*C. annuum*	SP71	0.140
P1034	*C. annuum*	SP80	0.073
P1035	*C. annuum*	SP96	0.076
P1036	*C. annuum*	SP98	0.106
P1037	*C. annuum*	SP100	0.153
P1038	*C. annuum*	SP104Y	0.081
P1044	*C. annuum*	CM334-1-22	0.094
P1050	*C. annuum*	Jalapeno (OP)	0.082
P0357	*C. baccatum* var. *pendulum*	00A00660	0.160
P0445	*C. baccatum* var. *praetermissum*	93A01929	0.183
P0583	*C. baccatum* var. *baccatum*	98A00685	0.102
P0011	*C. chinense*		0.096
P0653	*C. chinense*	94A05917	0.091
P1049	*C. chinense*		0.136
P0033	*C. frutescens*	00A05011	0.114
P0157	*C. frutescens*	04A00477	0.120
P0405	*C. pubescens*	04A02777	0.238
P0406	*C. pubescens*	04A02778	0.112
P0100	*C.* sp.	01A00456	0.082

seq 相關研究 (Zhang *et al*. 2010; Yamazaki *et al*. 2020)，本試驗共評估 2 種限制酶酵素組合 *Pst*I/*Msp*I 及 *Pst*I/*Mse*I，其中識別 6 個核苷酸的限制酶*Pst*I 為 rare-cutter，另一端則以識別 4 個核苷酸的限制酶 *Msp*I 或 *Mse*I 作為 common-cutter。兩種酵素組合皆以電腦模擬及活體外試驗進行評估比較，電腦模擬部分以 R 軟體 SimRAD (Lepais & Weir 2014) 進行，番椒參考基因體採用 CM334 品種 (Kim *et al*. 2014) v.1.6.0 (下載自 http://peppergenome.snu.ac.kr)。

ddRAD 文庫製備及定序

文庫製備依照Peterson *et al*. (2012) 及Kao (2016) 的方法，流程簡述如下：

(1) 雙重限制酶酶切 (digestion)：取 300 ng 的 gDNA，以 *Pst*I-HF®/*Msp*I 或 *Pst*I-HF®/*Mse*I (New England Biolabs, Ipswich, MA, USA) 於 37℃下進行酶切至少 4 h。

(2) 黏合銜接子 (adapter ligation)：將酶切完的 DNA 溶液加入 4 pmol P1 (針對 rare-cutter)、40 pmol P2 (針對 common-cutter) 銜

接子 (P1 及 P2 銜接子序列如表 2)，並以 6.6 Weiss units T4 DNA ligase (New England Biolabs, Ipswich, MA, USA) 及 1 mM rATP (Promega, Madison, WI) 於 16℃反應過夜，之後以 65℃ 20 min 使連接酶 (ligase) 失去活性。

(3) 純化 DNA：以 0.8× Ampure XP beads (Beckman Coulter Genomics, Brea, CA, USA) 去除 buffer 與酵素，並回溶至 42 μL 二次水。

(4) 連接標誌 (index) 及晶片 (flowcell) 連接序列：將帶有 Nextera-XT-Index-Kit2 (Nextera-XT-Index 序列如表 3 所示) 及 flowcell 黏合序列的引子 (0.5 μM)，以 0.02 U Phusion High-Fiedlity DNA Polymerases (Thermo Fisher Scientific, Waltham, MA, USA)、200 μM dNTPs 進行聚合酶連鎖反應，反應條件為 98℃ 30 s、98℃ 10 s、72℃ 30 s，18 cycles；72℃ 10 min；保存於 4℃。

(5) 聚合酶連鎖反應 (polymerase chain reaction; PCR) 產物純化並定量：同樣以 0.8× Ampure XP beads (Beckman Coulter Genomics, Brea, CA, USA) 去除 buffer 與酵素，回溶至 22 μL 二次水後以 qubit (Thermo Fisher Scientific, Waltham, MA, USA) 定量。

(6) 混合樣本並進行產物片段篩選：將不同 index 的 PCR 產物等體積混合，以 BluePippin 系統 (Sage Scientific, Beverly, MA, USA) 篩選 250–600 bp 的片段。

(7) 濃縮文庫並確認品質：以 1× Ampure XP beads (Beckman Coulter Genomics, Brea, CA, USA) 濃縮 DNA 文庫，濃度至少達 10 ng μL^{-1}，NanoDrop 品質檢測需達 260/280 = 1.8–1.9、260/230 > 1.8。

(8) 定序：以 HiSeq X™ Ten (Illumina, San Diego, CA, USA) 次世代定序儀進行定序，採 150 bp pair-end。

定序資料分析及 SNP 探勘

原始定序資料首先使用 FastQC 及 MultiQC 進行讀序品質及數量總結，接續以 Stacks v2.61 軟體的 process_radtag 程式移除品質不佳的讀序，如酵素切位不合、index 序列錯誤、讀序品質 Phred quality score 小於 20 者，再以 BWA-MEM v0.17.7 程式 (Li 2013) 將讀序對應至參考序列 *C. annuum* CM334 v1.60 組裝版本 (http://peppergenome.snu.ac.kr)，使用指令為 bwa mem -M {CM334_reference.fasta} {cleaned_forwaed.fastq} {cleaned_reverse.fastq} > {aligned_SAM}，並經 Picard v2.25.2 軟體 (https://broadinstitute.github.io/picard) 的 SortSam 與 MarkDuplicates 指令篩選可成對對應到參考序列 (properly paired) 且只對應到 1 個基因體上的位置 (uniquely mapped) 的成對讀序，使用指令為 SortSam I = {input} O = {output} SORT_ORDER = coordinate；MarkDuplicates I = {input} O={output} M = {output.metrics}。上述之成對讀序經 samtools v1.7 軟體 (Danecek *et al*. 2021) 的 view 指令確認讀序數量，指令為 samtools view -c -f 2 -F 2308 {input})，並使用 flagstat 或 mpileup 指令分析 BAM 檔案資料完後 (指令為 samtools flagstat {input}；samtools mpileup {input})，進入下一個步驟：SNP 探勘。SNP 探勘以 GATK v4.2.0.0 軟體 (https://gatk.broadinstitute.org/hc/en-us) 的 HaplotypeCaller 程式進行，先產生包含所有基因位點的 GVCF 中間檔案 (指令為 HaplotypeCaller -R {CM334_reference.fasta} -I {input} -O {output} -ERC GVCF")，再以 GenomicsDBImport 及 GenotypeGVCFs 指令將個別樣本的變異位點數據做統整、分析，建立樣本族群變異位點資料庫 (指令為 GenomicsDBImport --genomicsdb-workspace-path {output_path} --sample-name-map {sample_

表 2.　本研究使用之 P1 及 P2 銜接子序列。

Table 2.　The nucleotide sequences of the P1 and P2 adapters used in this study.

Name	Description	Sequence
Adapter-P1	Rare-cutter	5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTTGCA-3'
Adapter-P2	Common-cutter	5'-CGAGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3'

表 3. 本研究使用之 Nextera-XT-Index 序列。

Table 3. The nucleotide sequences of the Nextera-XT-Index used in this study.

		I7_Index		I5_Index	
Sample	Species	ID	Sequence	ID	Sequence
20F-P0011	*Capsicum chinense*	N701	TAAGGCGA	S513	CTAGTCGA
20F-P0033	*C. frutescens*	N702	CGTACTAG	S513	CTAGTCGA
20F-P0100	*C.* sp.	N703	AGGCAGAA	S513	CTAGTCGA
20F-P0133	*C. annuum*	N704	TCCTGAGC	S513	CTAGTCGA
20F-P0134	*C. annuum*	N701	TAAGGCGA	S515	AGCTAGAA
20F-P0157	*C. frutescens*	N702	CGTACTAG	S515	AGCTAGAA
20F-P0164	*C. annuum*	N703	AGGCAGAA	S515	AGCTAGAA
20F-P0287	*C. annuum*	N704	TCCTGAGC	S515	AGCTAGAA
20F-P0357	*C. baccatum*	N701	TAAGGCGA	S516	ACTCTAGG
20F-P0405	*C. pubescens*	N702	CGTACTAG	S516	ACTCTAGG
20F-P0406	*C. pubescens*	N703	AGGCAGAA	S516	ACTCTAGG
20F-P0445	*C. baccatum*	N701	TAAGGCGA	S517	TCTTACGC
20F-P0565	*C. annuum*	N702	CGTACTAG	S517	TCTTACGC
20F-P0583	*C. baccatum*	N703	AGGCAGAA	S517	TCTTACGC
20F-P0653	*C. chinense*	N704	TCCTGAGC	S517	TCTTACGC
20F-P1025	*C. annuum*	N701	TAAGGCGA	S518	CTTAATAG
20F-P1027	*C. annuum*	N702	CGTACTAG	S518	CTTAATAG
20F-P1028	*C. annuum*	N703	AGGCAGAA	S518	CTTAATAG
20F-P1032	*C. annuum*	N701	TAAGGCGA	S520	ATAGCCTT
20F-P1033	*C. annuum*	N702	CGTACTAG	S520	ATAGCCTT
20F-P1034	*C. annuum*	N703	AGGCAGAA	S520	ATAGCCTT
20F-P1035	*C. annuum*	N704	TCCTGAGC	S520	ATAGCCTT
20F-P1036	*C. annuum*	N701	TAAGGCGA	S521	TAAGGCTC
20F-P1037	*C. annuum*	N702	CGTACTAG	S521	TAAGGCTC
20F-P1038	*C. annuum*	N703	AGGCAGAA	S521	TAAGGCTC
20F-P1044	*C. annuum*	N701	TAAGGCGA	S522	TCGCATAA
20F-P1049	*C. chinense*	N702	CGTACTAG	S522	TCGCATAA
20F-P1050	*C. annuum*	N703	AGGCAGAA	S522	TCGCATAA
20F-P0033-mse	*C. frutescens*	N705	GGACTCCT	S513	CTAGTCGA
20F-P0434-mse	*C. annuum*	N705	GGACTCCT	S515	AGCTAGAA
20F-P1025-mse	*C. annuum*	N705	GGACTCCT	S516	ACTCTAGG
20F-P1028-mse	*C. annuum*	N705	GGACTCCT	S517	TCTTACGC

name_list.txt} -L {params.intervals} --reader-threads 20；GenotypeGVCFs 則為 -R {CM334_reference.fasta} -V gendb://{input.chr_file} -O {output})，接著以 Picard 軟體的 GatherVcfs 指令將分析數據整併成單一 VCF 檔案 (指令為 GatherVcfs I = {input.ch01} I = {input.ch02} I = {input.ch03} I = {input.ch04} I = {input.ch05} I = {input.ch06} I = {input.ch07} I = {input.ch08} I = {input.ch09} I = {input.ch10} I = {input.ch11} I = {input.ch12} O = {output})。最後使用 GATK 軟體的 SelectVariants 指令 (-R {CM334_reference.fasta } -V

{input} --select-type-to-include SNP -O {output_SNP.vcf}) 擷取 SNP 資料。獲得之 SNP 資料以 VCFtools v0.1.15 軟體 (Danecek *et al*. 2011) 篩選 SNP，設定參數為 --minDP 5 --minGQ 15 --max-missing 0.5，篩選後 VCF 檔案匯入 TASSEL v5.0 軟體 (https://www.maizegenetics.net/tassel) 進行總結。過程中以 Integrative Genomics Viewer (Thorvaldsdóttir *et al*. 2013) 及 R 語言 CMplot 套件 (https://CRAN.R-project.org/package=CMplot) 輔助讀序及 SNP 視覺化呈現。

主成分分析與親緣分析

主成分分析及親緣分析皆於 TASSEL5 進行。主成分分析設定最低維度參數為 2，搭配共變異數矩陣 (covariance matrix) 進行分析，而後將獲得的 PC1 以及 PC2 座標匯出為 tsv 格式，後續則使用 Past v4.03 軟體 (Hammer *et al*. 2001) 進行視覺化呈現，並標註每個樣本的對應位置。親緣分析則依據主成分分析 (principal component analysis; PCA) 的輸出資料，搭配 Past v4.03 軟體的聚類分析 (clustering) 功能繪製成樹狀圖，調整參數為非加權算術平均連鎖法 (unweighted pair-group method using the average approach; UPGMA) 進行相似度計算。

結果

比較不同限制酶組合用於番椒 ddRAD 定序

為選擇較適合番椒ddRAD的限制酶組合，本研究比較 2 種番椒簡化基因體定序之酵素組合—*Pst*I/*Msp*I 及 *Pst*I/*Mse*I。電腦模擬結果顯示，*Pst*I/*Msp*I 組合可產生 322,050 個片段，*Pst*I/*Mse*I 組合可產生 5,509,217 個片段，後者產生的片段數為前者的 17 倍，但多屬小片段 (95% 片段短於 403 bp)，而前者產生的片段長度則涵蓋較廣，95% 片段介於 0–7,467 bp (表 4)。而針對目標 150–500 bp 間的片段，*Pst*I/*Msp*I 組合及 *Pst*I/*Mse*I 組合分別產生為 54,209 及 1,268,219 個，前者片段大小分布較均勻，而後者有短片段較多的趨勢。再更深入針對目標 150–500 bp 間片段探討酵素酶切型態，發現片段兩端為不同酵素酶切者 (AB + BA，AB 代表 5′ 端以酵素酶 *Pst*I 切、3′ 端以酵素酶 *Msp*I 或 *Mse*I 切之讀序；BA 代表 5′ 端以酵素酶 *Msp*I 或 *Mse*I 切、3′ 端以酵素酶 *Pst*I 切之讀序)，*Pst*I/*Msp*I 組合及 *Pst*I/*Mse*I 組合各有 13,242 及 39,110 個，分別占全部 150–500 bp 目標片段之 24.4% 及 3.1% (圖 1)。片段兩端為相同酵素酶切的片段 (AA + BB，AA 代表兩端皆為酵素酶 *Pst*I 切之讀序；BB 代表兩端皆為酵素酶 *Msp*I 或 *Mse*I 切之讀序) 及 BA 形式酶切的片段，在 PCR 擴增階段因為 Y 型 adapter 的設計而不會被擴增，所以只有 AB 的片段可被擴增從而被定序，因此依據電腦模擬結果，*Pst*I/*Msp*I 組合及 *Pst*I/*Mse*I 組合分別得到 6,484 及 18,926 個可被定序的片段，分別為總片段之 2.01% 及 0.34%。

實際以 3 個樣本 (P0033、P1025、P1028) 觀察 *Pst*I/*Msp*I 及 *Pst*I/*Mse*I 組合之定序結果 (表 5)，*Pst*I/*Msp*I 組合比 *Pst*I/*Mse*I 組合得到較少讀序，前者為 20–32 M 讀序，後者為 35–38M 讀序，但經讀序品質過濾後，成對對應到參考序列 (properly paired) 且只對應到 1 個基因體上的位置 (uniquely mapped) 的讀序數量，反而 *Pst*I/*Msp*I 組合比 *Pst*I/*Mse*I 組合得到較多

表 4. 電腦模擬不同酵素組合對番椒參考基因體 (*Capsicum annuum* ‘CM334’) 酶切所得到之讀序數量。

Table 4. *In silico* simulation of reads number of pepper reference genome (*Capsicum annuum* ‘CM334’) digested with different restriction enzyme pairs.

Restriction enzyme pair	Total reads number	bp of 95% reads	Number of reads in 150–500 bp	Number of reads in 150–500 bp, AA + BB type	Number of reads in 150–500 bp, AB + BA type	Number of reads in 150–500 bp, AB type
*Pst*I/*Msp*I	322,050	7,467	54,209	40,967	13,242	6,484
*Pst*I/*Mse*I	5,509,217	403	1,268,219	1,229,109	39,110	18,926

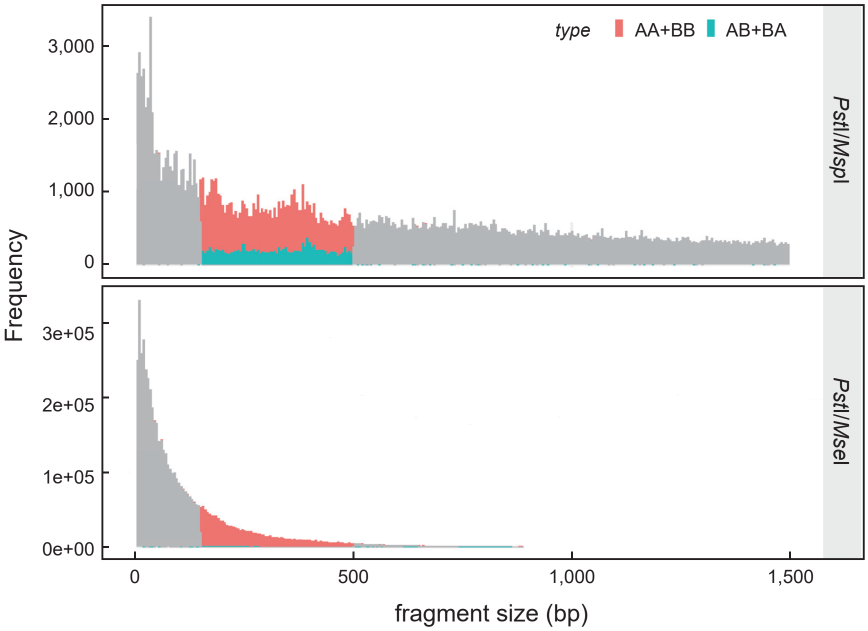

圖 1. 電腦模擬不同酵素組合對番椒參考基因體 (*Capsicum annuum* 'CM334') 酶切所得到之讀序分布情形。灰色區域表示酶切後所有讀序，紅色區域為長度介於 150–500 bp 且兩端為相同酵素酶切 (AA + BB) 之讀序，藍色區域為長度介於 150–500 bp 且兩端為不同酵素酶切 (AB + BA) 之讀序。AA：兩端皆為酵素酶 *Pst*I 切之讀序，BB：兩端皆為酵素酶 *Msp*I 或 *Mse*I 切之讀序，AB：5′ 端以酵素酶 *Pst*I 切、3′ 端以酵素酶 *Msp*I 或 *Mse*I 切之讀序，BA：5′ 端以 *Msp*I 或 *Mse*I 切、3′ 端以酵素酶 *Pst*I 切之讀序。

Fig. 1. *In silico* simulation of the read distribution came from digesting the *Capsicum annuum* reference genome ('CM334') with different pairs of restriction enzymes. Grey area: all the restriction fragments; red area: fragments in the range 150–500 bp and digested with the same restriction enzymes in both end; blue area: fragments in the range 150–500 bp and digested with different restriction enzymes in both end. AA: fragments digested with the same restriction enzymes *Pst*I in both end; BB: fragments digested with the same restriction enzymes *Msp*I or *Mse*I in both end; AB: fragments digested with restriction enzyme *Pst*I in 5′ end and restriction enzyme *Msp*I or *Mse*I in 3′ end; BA: fragments digested with restriction enzyme *Msp*I or *Mse*I in 5-end and restriction enzyme *Pst*I in 3′ end.

讀序，分別約為 19–28 M 及 15–17 M 條讀序，高品質讀序數量約為原始讀序數量的 87−92% 及 43−46%。比較兩個組合之讀序分布情形，由圖 2 可見，兩個組合之讀序皆涵蓋番椒 12 條染色體，且皆有讀序在染色體兩端較多的情形，其中 *Pst*I/*Mse*I 組合的讀序於各區域數量上落差較小，*Pst*I/*Msp*I 組合的讀序則在部分區域有讀序較多的情形。

番椒種原基因體分析

本研究第二部分針對 28 個番椒收集系以 *Pst*I/*Msp*I 酶切並進行 ddRAD 定序，平均每個樣本得到 26 M 條讀序，拼貼回番椒參考序列的總長度為 137,970,405 bp，約占全基因體 3.9%。經讀序品質篩選及探勘 SNP 等步驟後，共得到 850,400 個 SNP，其中深度達 5 以上、正確率 97% 以上、遺失率小於 50% 的高品質 SNP 共 14,230 個 (圖 3)。此些 SNP 涵蓋番椒 12 條染色體，平均每條染色體有 1,185 個 SNP，以第 1 條染色體有最多 SNP，第 8 條染色體有最少 SNP；12 條染色體平均 204 Kb 有 1 個 SNP，密度最高為第 2 條染色體，每 131 Kb 出現 1 個 SNP，最低為第 10 條染色體，約 256 Kb 才會出現 1 個 SNP (表 6)。

28 個番椒收集系以種的位階進行分類 (*C. annuum*、*C. baccatum*、*C. chinense*、*C. frutescens*、*C. pubescens* 及未知種 *C.* sp.) 並計算不同種番椒成對對應到參考序列且只對應到 1 個基因體上的位置的讀序數量，不同種番椒材料經 *Pst*I/*Msp*I 酶切並進行 ddRAD 定序

表 5.　不同酵素組合酶切得到的讀序數量。

Table 5.　Number of sequencing reads digested with different restriction enzyme pairs.

Enzyme pairs	Line	Total reads	Mapped reads	Uniquely mapped reads	Uniquely mapped and properly paired reads
*Pst*I-*Msp*I	P0033	25,729,102	23,606,978	23,398,772	22,362,212
		100%	92%	91%	87%
	P1025	32,647,308	29,575,297	29,421,077	28,613,694
		100%	91%	90%	88%
	P1028	20,797,859	19,842,359	19,717,124	19,204,094
		100%	95%	94%	92%
*Pst*I-*Mse*I	P0033	35,753,621	28,320,981	23,979,588	15,586,808
		100%	79%	67%	43%
	P1025	37,621,037	29,687,317	25,363,306	16,913,924
		100%	79%	67%	45%
	P1028	37,924,974	30,312,547	25,920,205	17,507,400
		100%	80%	68%	46%

所得可進行後續 SNP 分析之讀序數目約占原始讀序數目之 75.6–93.9% (圖 4)。

28 個番椒收集系來自 5 個番椒馴化種，異質度 (heterozygous proportion) 介於 7.1–23.8%，平均為 11.2% (表 1)。觀察不同種樣本的異質度，並無顯著偏高或偏低的情況；再深入觀察自交系 (名稱為 SP 開頭) 與種原 (名稱為作物種原庫編碼 xxAxxxxx) 的異質度，亦無顯著何者較高或較低的情況。

番椒種原集群分析

以 28 個番椒收集系之 14,230 個 SNP 進行主成分分析，結果顯示約可分為 4 群，其中 *C. baccatum* 及 *C. pubescens* 可各自清楚地被劃分為一群，分別位於圖 5 左下角及左上角，17 個 *C. annuum* 品系及 1 個未知品系 (*C.* sp.) 則皆位於圖 5 右側且相當集中，*C. chinense* 及 *C. frutescens* 於圖正中央被劃分為同一群，不過有 1 個 *C. chinense* 及 1 個 *C. frutescens* 也被歸類至 *C. annuum* 一群。

非加權算術平均連鎖法 (UPGMA) 的結果 (圖 6) 顯示，*C. pubescens* 及 *C. baccatum* 的品系最早被分開，分別為 Group I 及 Group II，*C. annuum*、*C. chinense*、*C. frutescens* 及 sp. 的品系位於同一分支 (Group III)，此分支若再細分，*C. chinense* 及 *C. frutescens* 的部分品系可再區分為一分支 Group III-a，*C. annuum* 及 *C.* sp. 則全數落於 Group III-b。此結果與前述主成分分析結果一致。

討論

簡化基因體定序利用限制酶酵素剪切或物理震盪方式，將定序資源集中少部分基因體，因此在經費有限的前提下，對於基因體較大的作物，簡化基因體定序為相當合適的定序方法。番椒的基因體相當大 (3–3.5 Gb)，為同屬茄科的番茄 (950 Mb)、馬鈴薯 (844 Mb) 、茄子 (1.13 Gb) 基因體的 3 倍以上，且其基因體組成亦相當複雜，約 75–80% 由 repetitive elements 組成 (Hulse-Kemp *et al*. 2018)。有鑑於此，本研究擬以簡化基因體定序方法進行番椒種原基因體資訊分析，以降低定序成本，且相比於常見使用 1 個酵素酶切或物理剪切方式，本研究採用 2 個酵素酶切，使得在相同定序通量下，定序片段更加集中部分區域，提升基因體資料的正確率 (Peterson *et al*. 2012)。

酵素的選擇會影響到基因體的二次抽樣 (subsampling)，導致酶切後片段數目、大小不同，從而影響到讀序及 SNP 的數量及分布 (Cumer *et al*. 2021)。在本研究中，第一部分探討常見用於番椒的雙酵素酶切組合 *Pst*I/*Msp*I

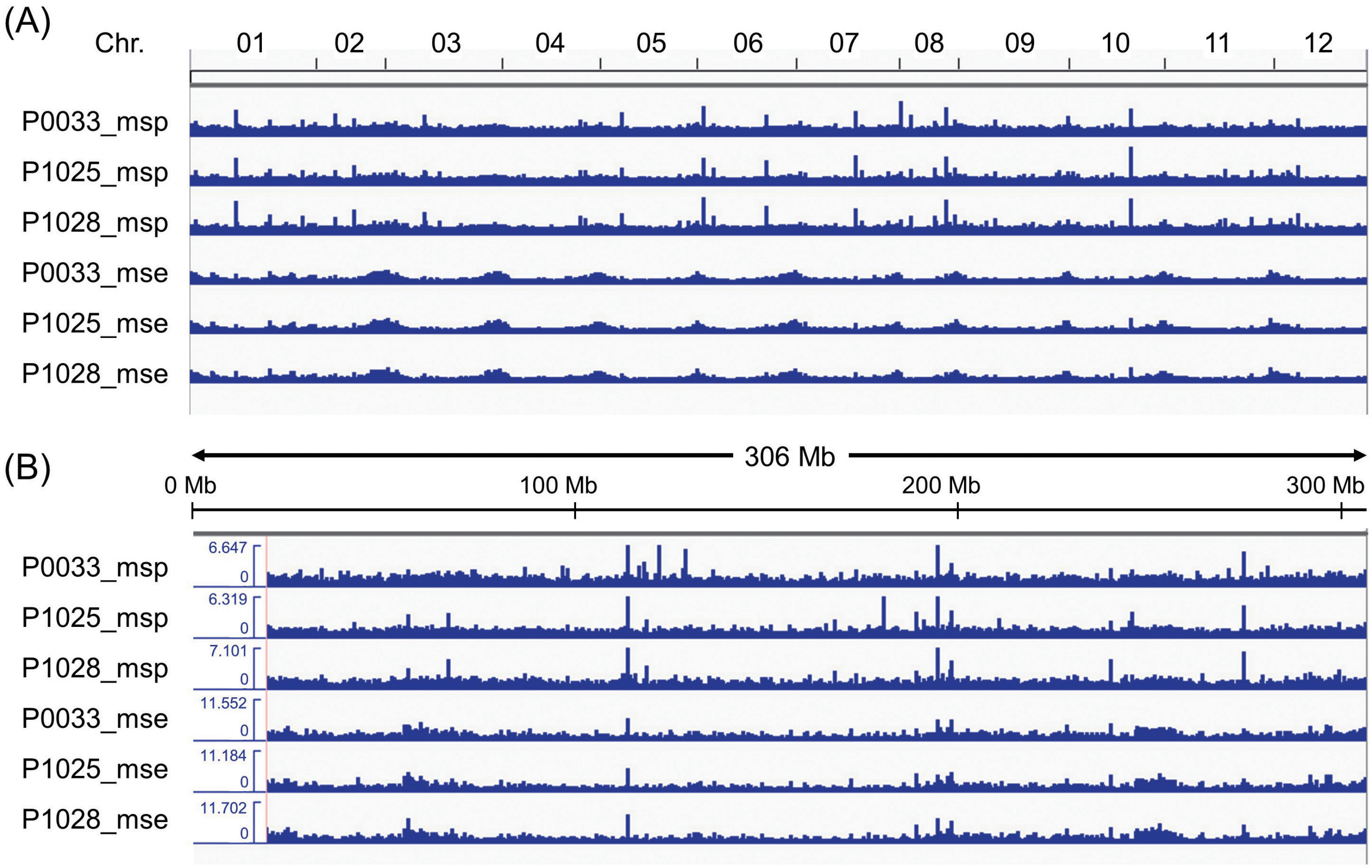

圖 2. 不同酵素組合針對相同樣本酶切所得到讀序之深度與分布概況。(A) 番椒 12 條染色體；(B) 番椒第一條染色體。X 軸代表染色體位置，Y 軸代表該區域之深度讀值。

Fig. 2. Distribution of the read depths with 6 restriction enzymes and pepper varieties combinations (A) along 12 chromosomes and (B) on chromosome 1. X axis represents the location of chromosome, and Y axis represents the reads depth of the region.

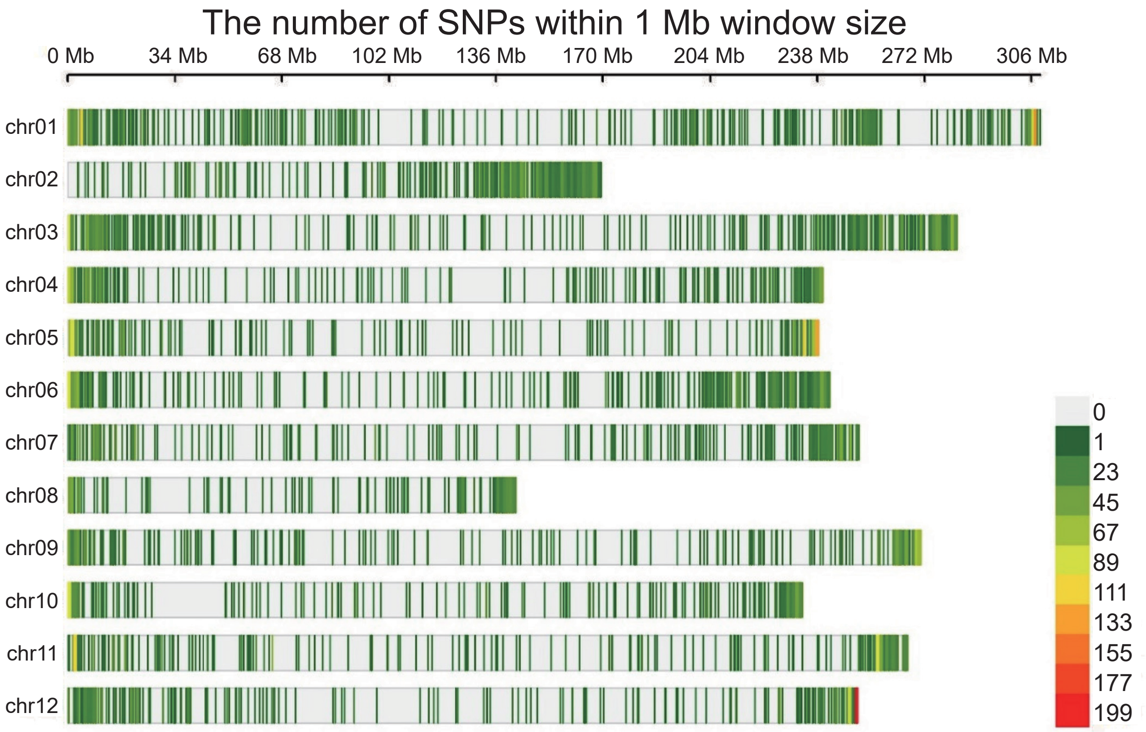

圖 3. 28 個番椒收集系的 14,230 個高品質單一核苷酸多型性位點分布。位點是使用 *Pst*I/*Msp*I 雙酶切的簡化基因體定序技術所產生。

Fig. 3. Distribution of 14,230 high-quality single nucleotide polymorphisms (SNPs) derived from the *Pst*I/*Msp*I double-digested RADseq technique for 28 pepper accessions.

表 6. 平均每條染色體 SNP 數量及密度。

Table 6. Number and density of single nucleotide polymorphism (SNP) per chromosome.

Chromosome	Number of SNP	Average distance (bp) between SNP
1	1,840	167,990
2	1,296	130,830
3	1,685	167,822
4	999	240,361
5	1,099	216,875
6	1,353	179,040
7	1,075	233,761
8	582	244,616
9	1,089	248,928
10	910	256,398
11	1,130	236,168
12	1,172	214,104
Average	1,186	203,655

及 *Pst*I/*Mse*I，何者較適宜用於番椒 ddRAD 定序。電腦模擬結果顯示，*Pst*I/*Mse*I 產生的片段數雖為 *Pst*I/*Msp*I 的 17 倍，但大部分皆為短片段，在目標片段選拔的階段會被移除高比例的片段，較浪費建庫資源；從可被定序片段的數量來看，*Pst*I/*Msp*I 及 *Pst*I/*Mse*I 分別得到 6,484 及 18,926 個可被定序的片段，為總片段之 2.01% 及 0.34%，此亦顯示 *Pst*I/*Msp*I 組合在建庫過程中資源較不浪費。

以實際 2 個酵素組合的定序結果來看，得到的讀序數量 *Pst*I/*Mse*I 多於 *Pst*I/*Msp*I 組合，與預期 (電腦模擬) 結果一致，但經過對應回參考序列 (alignment) 的步驟後，成對對應到參考序列 (properly paired) 且只對應到 1 個基因體上的位置 (uniquely mapped) 的讀序數量，卻以 *Pst*I/*Msp*I 組合 (19–28 M) 比 *Pst*I/*Mse*I 組合 (15–17 M) 得到較多讀序，分別約

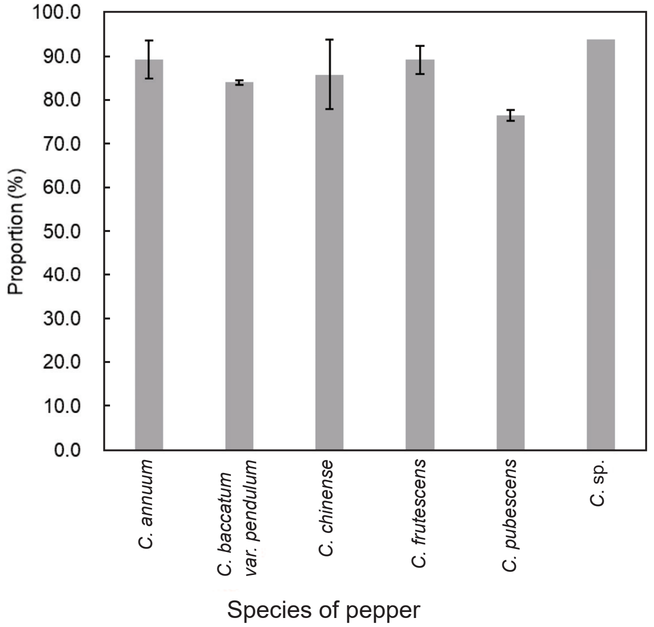

圖 4. 28 個番椒收集系以種的位階進行分類 (*Capsicum annuum*、*C. baccatum*、*C. chinense*、*C. frutescens*、*C. pubescens* 及未知種 *C.* sp.)，計算不同種番椒材料成對對應到參考序列 (properly paired) 且只對應到 1 個基因體上的位置 (uniquely mapped) 的讀序數量。誤差線為標準差 (*C.* sp. 僅有 1 個樣本故未計算與標示標準差)。

Fig. 4. 28 accessions were classified by *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, *C. pubescens* and *C.* sp. into 6 groups. The corresponding properly paired and uniquely mapped reads for each group were calculated. Error bar: standard deviation (SD) (Only 1 sample of *C.* sp. was included, no SD for *C.* sp. was calculated and labeled).

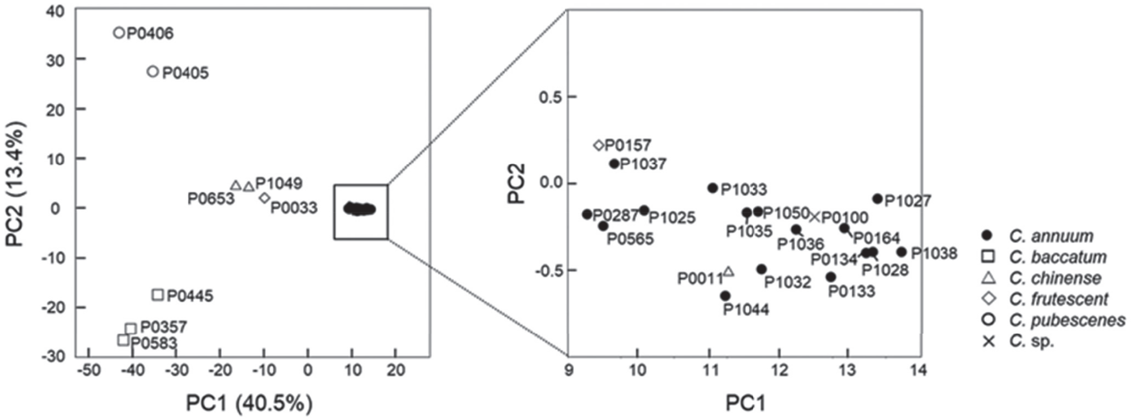

圖 5. 28 個番椒收集系之主成分分析圖。以 14,230 個 SNP 資料所計算，每個點表示不同品系，點的顏色表示不同種。X 軸與 Y 軸分別代表 PC1 (40.5%) 與 PC2 (13.4%)。

Fig. 5. The principal component analysis (PCA) plot of 28 pepper accessions using genotypes of 14,230 single nucleotide polymorphisms (SNPs). Each point represents the SNP profile of an individual. The color represents *Capsicum* species. X axis represents the variance explained by PC1 (40.5%), and Y axis represents the variance explained by PC2 (13.4%).

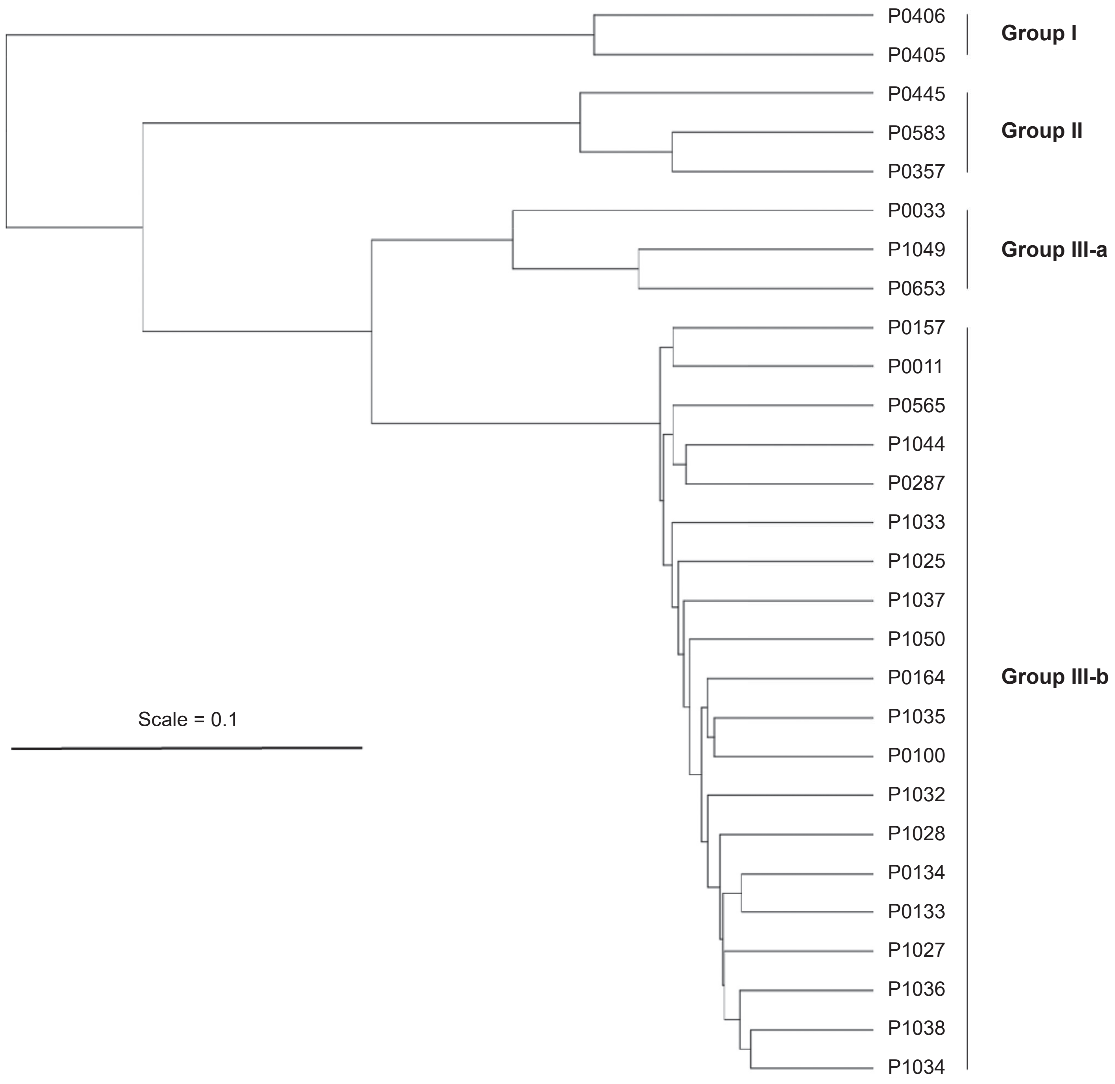

圖 6. 28 個番椒收集系之親緣關係樹。此圖是使用 28 個番椒在 14,230 個單一核苷酸多型性位點的基因型所建構。

Fig. 6. Rooted phylogenetic tree based on the genotypes of 14,230 single nucleotide polymorphisms (SNPs) of the 28 *Capsicum* accessions.

為原始讀序數量的 87–92% 及 43–46%。此顯示在相同初始 gDNA 質量及相同建庫方法下，*Pst*I/*Msp*I 可得到較多成對且比對專一的讀序。

本研究測試的 2 組酵素 *Pst*I/*Msp*I 及 *Pst*I/*Mse*I，其 rare cutter 皆為 *Pst*I，屬甲基化敏感 (methylation-sensitive) 的限制酶，另一端 (common cutter) 則分別為甲基化敏感的 *Msp*I 及甲基化不敏感 (methylation-insensitive) 的 *Mse*I。前人研究顯示甲基化敏感的限制酶能更有效針對富含基因的區段進行酶切，從而降低基因體複雜度 (減少 repeat element) (Fellers 2008; Zhang *et al*. 2010)。Pootakham (2016) 等人針對油棕的基因體研究更實際指出，使用 2 個非甲基化敏感的限制酶時，僅 34% 的 SNP 位在基因上或附近 (5 Kb)，同時使用 2 個甲基化敏感的限制酶時，則有 60–67% 的 SNP

落於基因上或附近。考量 *Pst*I/*Msp*I 組合可更集中定序資源於基因豐富的區域，且不論在建庫資源利用上或是成功對應回參考序列的讀序數量/比例上，*Pst*I/*Msp*I 皆較 *Pst*I/*Mse*I 表現佳，爰此，本研究第二部分將以 *Pst*I/*Msp*I 組合進行番椒種原 ddRAD 建庫，並進行番椒種原遺傳背景分析。

本研究第二部分針對 28 個分屬 5 個馴化種的番椒收集系，以 *Pst*I/*Msp*I 酶切並進行 ddRAD 定序，共得到 850,400 個 SNP，經篩選過後以 14,230 個高品質 SNP 進行後續番椒種原基因體初探。如圖 4，在 28 個番椒收集系中，不同種番椒材料經酶切及定序所得可成對對應到參考序列且只對應到 1 個基因體上的位置讀序數目約占原始讀序數目之75.6–93.9%，大致上以 *C. pubescens* 稍低 (小於 80%)，其餘均高於 80%，說明不同種番椒之間之基因體組成與序列仍具有差異，然而無論有效讀數占比在不同種番椒之間具有差異，後續仍可進行 SNP 分析。另外，相比於其他有關番椒種原 (涵蓋 5 個馴化種) 簡化基因體定序的文獻，Ramchiary & Kole (2019) 使用 14,444 個 SNP、Pereira-Dias *et al*. (2019) 使用 4,083 個 SNP、Potnis *et al*. (2019) 使用90,794個SNP，由於每篇研究使用的建庫方法、定序方法、SNP 篩選條件等皆不同，因此在 SNP 數量上無法進行比較，不過以 SNP 於番椒基因體分布情形來看，本研究得到的 14,230 個高品質 SNP 涵蓋全部染色體，並與預期一致地以染色體兩端基因較密集處較多，染色體中段亦無明顯缺失 SNP 處，因此這些 SNP 應可用於番椒基因體分析。為初步瞭解此些 SNP 用於跨種番椒基因體分析是否合適，因此本研究進一步將此些 SNP 對不同種番椒進行集群分析。

以 28 個分屬 5 個馴化種的番椒收集系之 14,230個SNP進行集群分析，主成分分析圖及樹狀圖皆顯示 *C. baccatum* 及 *C. pubescens* 的品系可各自為一分群或一分支，*C. annuum*、*C. chinense* 及 *C. frutescens* 則先分到同一分支之後，才又將*C. annuum*、*C. chinense*及*C. frutescens* 分開，而 *C. chinense* 及 *C. frutescens* 則較難區分。前人研究以性狀與分子標誌技術將番椒種類分成 3 大群，其中 *C. annuum*、*C. chinense* 及 *C. frutescens* 因親緣性接近，合稱為 *C. annuum* complex，另外2種 *C. baccatum* 及 *C. pubescens* 在分類上差異大，故分別以 *C. baccatum* complex 及 *C. pubescens* complex 稱呼 (Walsh & Hoot 2001; García *et al*. 2016; Shiragaki *et al*. 2020)。本研究分群結果與前人研究一致，不過有 2 個品系的分群結果與原先種原資料不符，P0011 (*C. chinense*) 與 P0157 (*C. frutescens*) 被分到 Group III-b (多為 *C. annuum*)，可能原因為已與 *C. annuum* 雜交，或是原先種原資料登記錯誤，實際觀察分類上重要特徵，如：花瓣色、花藥色、萼頸環縮 (caylx annular constriction) 及萼緣齒突 (teeth) 等，亦無法清楚分辨此二品系之種名。本研究 5 個馴化種的集群分析結果與前人研究相符，說明本研究使用的一系列建庫、定序及生物資訊分析等方法可作為跨種番椒種原基因體分析之用。

結論

次世代定序技術已廣泛用於作物基因體研究，亦延伸出許多相關技術，然而每個作物適宜的建庫方法不同，有必要針對目標作物先進行方法可行性測試及比較。本研究目標作物為番椒種原，由於種原涵蓋不同種且通常有收集系內仍有變異的情形，使得複雜度又比常見的雙親本族群更高，此外番椒基因體亦相當大，在定序上須更大量經費，因此本研究以雙重酶切 DNA 定序技術降低番椒基因體複雜度，同時並節省定序資源。本研究比較不同限制酶組合所產生的資料，並用於番椒種原集群分析，結果顯示本研究採用的建庫、定序、生物資訊分析等方法，可將番椒種原正確分類，此說明 *Pst*I/*Msp*I 雙重酶切 DNA 定序方法為跨種番椒基因體分析之良好工具。未來將以此為基礎，針對台灣番椒種原建立其基因體資訊，作為台灣番椒育種的根基。

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The Application of Double-Digest Restriction-Site Associated DNA Sequencing Technology in Pepper Germplasm

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Abstract

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Next-generation sequencing technologies lead to a new era of crop genetic research. To lower sequencing costs for building a single nucleotide polymorphism (SNP) map of a genome, the reduced representation genome sequencing technology was used to sequence the flanking DNA sequences of selected restriction enzyme sites of DNA samples. However, appropriate parameters for constructing the reduced representation sequencing library, such as the choice of restriction enzyme and sequencing depth, differ in target crops and population composition. As a result, methodological adjustments toward target crops and population composition are vital to maximizing the efficiency of sequencing resources. In this study, we presented a tailored double-digest restriction site-associated DNA sequencing (ddRAD-seq) genotyping method for pepper germplasm. In the first part of this work, the performance of two restriction enzyme pairs- *Pst*I/*Msp*I and *Pst*I/*Mse*I were assessed, and results of *in silico* simulation showed 2.01% and 0.34% fragments of the total digested fragment were sequenced, respectively. According to sequencing results, more uniquely mapped and adequately paired reads (19–28 M) were observed in *Pst*I/*Msp*I pair than in *Pst*I/*Mse*I (15–17 M). Therefore, in terms of resource utilization in library preparation, the enzyme pair *Pst*I/*Msp*I performed better than *Pst*I/*Mse*I. In addition, the number or ratio of reads that came from *Pst*I/*Msp*I digestion was more efficiently mapped to the reference genome. As for the second part of this work, the SNP maps of 28 pepper accessions belonging to 5 domestic species were constructed using the ddRAD-seq genotyping technique with *Pst*I/*Msp*I restriction enzyme combination. A total of 14,230 high-quality SNP were obtained and distributed evenly on the 12 chromosomes of pepper. Furthermore, as expected, the 28 pepper accessions were clustered into proper phylogenetic groups based on the principle component analysis and the dendrogram analysis. In summary, the ddRAD-seq genotyping technique was tailored to construct the SNP maps of the pepper germplasm in this study.

Key words: Reduced genome sequencing, Double-digest restriction enzyme, *Capsicum*, Genome analysis.

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盆花文心蘭生理檢測模式之建立

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摘要

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文心蘭盆花品種繁多，花形及花色多樣性多來自於屬間或種間雜交，其生育特性之歧異度常造成生產管理上之困擾，而少量引入試種的方式更是耗費生產成本，於適種品種的篩選效率上亦差。本研究針對文心蘭盆花品種 (系) 自行設計檢測系統進行生理特性檢測據以推估植株最適栽培環境條件，並同時以不同環境場域實際栽培作為比較。檢測結果受測品種 *Colmanara* Massai 'White' 為日夜溫 25/25℃均溫型、光強度約 200 μmol m^{-2} s^{-1}。*Miltonidium* Rosy Sunset 為日夜溫 25/25℃均溫型，光強度約 400 μmol m^{-2} s^{-1}。*Odontobrassia* Kenneth Biven 'Santa Barbara' 為日夜溫 26/20℃，光強度約 300 μmol m^{-2} s^{-1}，檢測結果與實際場域栽培結果相符，以反應曲面為基礎之生理檢測系統所呈現的特性趨勢，可作為栽培管理調整時的參考依據，同時也可大幅縮短業者試種摸索時間，加快業者篩選試種品種速度。

關鍵詞：反應曲面法、文心蘭、全株量測、光合作用。

前言

文心蘭亞族 (Oncidinae Subtribe) 原生於中南美洲全境，氣候由熱帶雨林至與草原交界的闊葉林帶，分布範圍從海平面到高海拔的冷涼地區，約有 600 種 (Karasawa 1989)。

文心蘭依生長習性主要為附生蘭及少數地生蘭，依葉片型態可分為厚葉系、棒狀葉系、扇型葉系、具假球莖厚葉系及具假球莖薄葉系等五種類，大小及葉姿均具不同變化，而不同葉片系統間植株的生育習性並不相同，現行商業栽種以具假球莖薄葉系為主流。除了葉型上的差異外，在分類學上文心蘭屬 (*Oncidium*) 為樹蘭亞科下最大的一屬，約有 300 個原生種，齒舌蘭屬 (*Odontoglossum*) 約 60 個原生種，蜘蛛蘭屬 (*Brassia*) 約 40 個原生種，其他另有堇花蘭屬 (*Miltonia*)、凹唇蘭屬 (*Comparettia*) 及茹氏蘭屬 (*Rodriguezia*) 等 (Senghas 2002)。除各屬不同種外，自然或人為的屬間雜交，創造出各種不同花色，花徑涵蓋了小輪、中輪及大輪花等各種不同類型，觀賞期長，部分品種更具香氣。多種的屬間遺傳背景，造就品種的多樣性，但卻使得文心蘭盆花的生長特性更為多樣，也更加難以掌握這些新興品種的生育特性。

台灣蘭花早期受限於裸根才能出口，剝除介質所造成的物理損傷、於貯運時的黑暗環境及失水，導致植株存活率低且恢復期長 (Lin & Lin 2005)。自 2016 年起美國核准台灣的文心蘭盆花可附帶介質輸美，對於文心蘭盆花產業來說，無疑是一項利多。然文心蘭盆花商業品種繁多，相較於已發展蓬勃多年的切花產業，盆花的生育特性及相關栽培管理知識相對缺乏，品種間對於生長環境的需求差異甚大。若能依據不同品種的生理特性進行分群，設定明確的栽培條件，便可依品種需求提供適當栽

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培環境，提升植株品質的穩定性與縮短生長時間；相關資料的建立，亦可加深進口國後續栽培業者對台灣盆花文心蘭的信心。

台灣雖在蘭花育種能力強，但對於如何提供適合栽培及開花等環境條件及品種的生長特性卻不夠瞭解 (Chen & Lin 2012)，無法提供國內外後續接手栽培的廠商甚至終端消費者完整的栽培資訊。Chen *et al*. (2012) 指出，在不同環境條件下栽培的植株，其性狀之表現即可能有所不同。針對此問題，行政院農業委員會農業試驗所花卉研究中心以光合作用過程中，植物在不同生長環境下的氣體交換特性 (Shen *et al*. 2010) 為基礎，搭配反應曲面分析法 (respond surface methodology; RSM) (此分析法係結合統計方法中的迴歸分析、實驗設計及數據契合法，依試驗所得的數據，建構系統的函數關係) (Chang 2002) 進行蘭花生理檢測技術之開發。檢測系統的核心概念，係以非破壞性的方式，連續監測受測植株在各環境條件下其光合作用能力的變化，因二氧化碳 (CO_2) 為光合作用時的必要原料，所以可透過 CO_2 的變化量反推光合作用效率。開放式氣體循環檢測，可以應用於長期的氣體變化量測，並與單一葉片光合作用量測方法互補。利用複合量測箱，更可以重複量測或是同時比較不同環境或栽培處理對於作物生育因子之影響。(van Iersel & Bugbee 2000)，所以光合作用研究者常針對實驗需求自行設計適合的系統 (Akers *et al*. 1985; Knight *et al*. 1988; Dai 1991; Miller *et al*. 1996; van Iersel & Bugbee 2000; Klingeman *et al*. 2005; Chen & Lin 2012; He & Li 2014)。由於影響光合作用的環境因子繁多，逐一檢測除了拉長試驗時間外，同時植株生長勢差異亦增加實驗誤差。RSM 是一結合統計分析與實驗設計的技術，透過有系統的實驗，搜尋反應的最適化條件 (Wu 2008)，具有下列優點：(1) 減少試驗次數，當條件因子愈多時，效果愈顯著；(2) 可同時探討數個因子，及因子間的交感作用，可知悉各因子對反應值的貢獻程度；(3) 藉由曲面圖，可快速得知最適化條件。其原理是利用實驗設計來進行有系統的條件挑選，再依控制條件 (自變數 x) 與反應值 (依變數 y) 來建立關係函數 $y = f(x_1, x_2, \cdots, x_n) + \varepsilon$ (ε 為反應值的誤差)，藉由函數在一定條件範圍內預測出來的結果，繪製成反應曲面圖協助判斷最適條件的範圍 (Liu & Peng 2002)。透過定量的試驗結果預測條件範圍內達成最適結果的條件，取代逐一試驗，可以用最少的試驗次數來達成目的 (Su 2005)。此法目前已廣泛應用在食品發酵、化學工業及生物科技等領域 (Chen & Peng 1996; Liu & Peng 2002; Hsu & Hung 2005; Liyana-Pathirana & Shahidi 2005; Wu 2008; Chiu *et al*. 2013)。

本研究之目的在於利用自行設計的檢測設備並配合 RSM 之應用，針對文心蘭盆花品種進行生理特性檢測，推估植株最適栽培環境條件，同時以不同環境場域實際栽培作為比較評估方法學的可行性，期望檢測資料可提供並協助國內業者乃至於國外接力栽培的廠商，確認各品種較適當的栽培環境條件，提升種苗品質穩定度。

材料與方法

文心蘭盆花品種材料及栽培環境設定

試驗所使用的文心蘭盆花品種如下：*Colmanara* Massai ‘White’、*Miltonidium* Rosy Sunset 及 *Odontobrassia* Kenneth Biven ‘Santa Barbara’，共 3 個品種，分別購自明翠谷蘭園 (台灣南投縣)、王世杰先生及三欣園藝 (台灣雲林縣)，植株規格統一為 2.5 吋 1 球帶 1 未展葉新芽，葉芽抽出約 5 cm。植株放置於日/夜溫約為 32/27℃的溫室中馴化，1 wk 後分批進行檢測。

葉面積之估算，參考 Lin & Lee (1988) 方式。取新芽形成假球莖後由上向下之第 2 葉，共 10 株，量測葉片最大長度 (基部至葉尖長度) 以及最大寬度，並以葉面積儀 (LI-300A, LI-COR Inc., Lincoln, NE, USA) 實際量測葉面積，帶入算式求得 K 值為 0.77，得到葉面積估算值 (cm^2) = 長 × 寬 × 0.77。將實際平均葉面積與估算平均葉面積值進行直線迴歸，其迴歸方程式之係數及決定係數 (R^2) 等在統計分析上皆具存在意義 (圖 1)，表示利用所建立之估算模式估計而得之葉面積估算值與實際葉面積頗為接近，故試驗葉面積皆以該方式估算。量

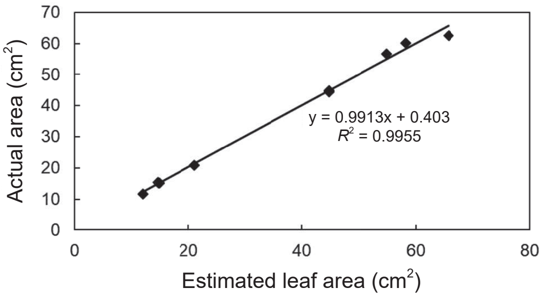

圖 1. 以共同常數估值估算之葉面積與實際葉面積之直線迴歸。

Fig. 1. Relationship between actual and pooled estimated leaf area (max. length × max. width × 0.77) for examining the adequacy of the estimated model.

取總葉面積後再平均分組，減少因葉面積差造成的組間影響。

文心蘭盆花品種營養生長最佳需求條件測試

光合作用效率採用全株 CO_2 交換量為監測依據，檢測系統參考 Ciou *et al*. (2015) 方法，再依文心蘭作物需求及儀器特性進行調整，試驗條件日溫、夜溫及光強度之設定範圍如下：日溫 (D) 25–30℃，夜溫 (N) 20–25℃，光度 (L) 200–400 μmol m^{-2} s^{-1}。

整體系統設計為，以密閉型壓克力箱 (內徑長 × 寬 × 高約 = 60 cm × 50 cm × 30 cm) 作為植株試驗空間，並連續監測壓克力箱內進氣端 CO_2 濃度與出氣端 CO_2 濃度變化。為達到試驗期間所需之不同日溫、夜溫與光度需求，壓克力箱置於生長箱 (FH-130, HIPOINT Company, Kaohsiung, Taiwan) 內，透過生長箱間接控制試驗所需的溫度、濕度及光照，溫度控制精度 ± 0.5℃ (25℃時)，濕度控制精度 ± 3% (80%，25℃時)，光週期為 12 h，日夜變化轉換期設定 1 h 之內模擬日照平均漸亮及漸暗。光度系由生長箱內燈管開啟數量進行調整，光度值以光度計 (LI-1400, LI-COR, Inc., Lincoln, NE, USA) 測量壓克力箱中間位置，監測植株最上位葉高度的光強度。

本試驗設計需長時間監測 CO_2 變化，故採用開放式氣體循環系統，為避免因人為活動對大氣 CO_2 濃度造成的影響，取氣口的位置位於溫室外，距地面高約 5 m 處，以抽氣馬達 (ACO-818, Zhejiang Sensen Industry Co., Ltd, Ruian, China) 經流量計 (flow meter, RMA-22-SSV 與 RMA-26-SSV, Dwyer Instruments, Inc., Michigan, IN, USA) 將氣體送入生長箱內，進入箱內的流率約 13–15 L min^{-1}。再以馬達 (TA-318 與 TA-388D, TAKAOKA, Tokyo, Japan) 抽取生長箱及壓克力箱空氣，流率流量分別控制在約 2 及 9 L min^{-1}，以二氧化碳/水汽分析儀 (CO_2/H_2O analyzer, LI-840A, LI-COR, Inc., Lincoln, NE, USA) 測定二氧化碳濃度。

影響植物生理的因子繁多，考量目前溫室可以有效控制的環境因子後，選定日溫、夜溫及光強度為本試驗主要檢測因子，探討對各品種最適環境條件組合。利用 Design-Expert 軟體 (DX10, V10.0.0.3, Stat-Ease, Inc. Minneapolis, MN, USA)，採用中央合成設計 (central composite design; CCD) 組合試驗條件，試驗變數及位準設計如表 1。試驗條件及組合中有 8 組複因子試驗點及 6 個軸上點，中心點採 4 重複，合計有 18 組試驗條件 (表 2)。每組試驗條件，以 5 株為 1 組置放於同一生長箱壓克力箱中，每次試驗前 1 d 均充分給水，每

表 1. 試驗變數及位準設計。

Table 1. Coded levels for the independent variables.

	Level		
Independent variable	Symbol	Coded	Uncoded
Day temperature (℃)	D	-1.68	26
		-1.00	28
		0.00	31
		1.00	33
		1.68	35
Night temperature (℃)	N	-1.68	21
		-1.00	23
		0.00	26
		1.00	28
		1.68	30
Light intensity (μmol m^{-2} s^{-1})	L	-1.68	80
		-1.00	125
		0.00	185
		1.00	225
		1.68	300

表 2. 試驗設計及條件組合。

Table 2. The arrangement and responses of central composite design.

No.	Day (℃)	Night (℃)	Light (μmol m^{-2} s^{-1})
1	30.0	25.0	400
2	30.0	20.0	400
3	23.3	22.5	300
4	25.0	20.0	200
5	27.5	22.5	468
6	30.0	25.0	200
7	27.5	18.3	300
8	31.7	22.5	300
9	30.0	20.0	200
10	25.0	25.0	200
11	27.5	22.5	300
12	25.0	25.0	400
13	27.5	22.5	300
14	27.5	26.7	300
15	27.5	22.5	132
16	25.0	20.0	400
17	27.5	22.5	300
18	27.5	22.5	300

4 min 記錄 1 次數據，累計計算淨二氧化碳交換量，單位葉面積之光合作用速率依 Long & Hällgren (1985, 1993) 所提之方法計算。

文心蘭盆花品種營養生長實際栽培測試

相同品種規格之植株，同時放置於台中新社溫室及雲林古坑網室兩處設施進行實際栽培，作為業者試種栽培模擬，調查其生長達外銷規格 (2 球 1 芽，芽體高度達前一代 1/3 高) 所需時間，以及假球莖之大小。每品種每區域各 15 株，並放置光度溫度紀錄器 (HOBO Data Loggers, Onset Computer Corporation, Pocasset, MA, USA) 記錄栽培全時期之環境，試驗調查時間為 2016 年 6 月至 2017 年 5 月。

結果

以日溫、夜溫及光強度為檢測條件利用 RSM 來量測不同環境下植物二氧化碳氣體交換能力以評估生理特性需求，此方法於蝴蝶蘭品種檢測上已獲得驗證 (Ciou *et al*. 2015)。然本研究所使用之文心蘭盆花均為帶有假球莖複莖型品種，與蝴蝶蘭為單莖無假球莖型蘭花不同，其綠色之新舊假球莖是否會干涉品種特性預測結果為主要關鍵。Hew & Yong (1994) 發現，文心蘭切花品種 *Oncidium* Goldiana 的假球莖雖含有高量的水分以及葉綠素，但因其外表附有角質層，因此無論是光照或是黑暗環境下，均無法觀察到有明顯的氣體交換現象。故本研究於計算產生氣體交換的總面積上，將假球莖忽略不計。

文心蘭盆花品種營養生長最佳需求條件測試

C. Massai ‘White’ 品種變方分析表中日溫 (P = 0.0059) 與夜溫 (P = 0.01) 皆達到顯著水準，其中又以日溫影響較大，顯示該品種對於溫度較為敏感，而光強度對於該品種無顯著影響之影響則較低 (P = 0.9465)，顯示該品種對於光度要求範圍較廣 (表 3)，於反應曲面圖的表現上，於 200–400 μmol m^{-2} s^{-1} 不同光度下，反應曲面趨勢以及光合作用效率的數值皆無顯著變化，但仍以 200 μmol m^{-2} s^{-1} 光度下之光合作用效率最高，溫度表現上，則皆可觀察到以日夜溫皆為 25℃的均溫環境下所展現的光合作用效率最高，且當日溫愈高，光合作用效率明顯變差，於日夜溫差變化上，光合作用效率亦會隨著日夜溫差加大而下降 (圖 2)，由以上結果，推論 *C.* Massai ‘White’ 之最適生長環境條件為光度 200 μmol m^{-2} s^{-1}，日夜溫環境條件則為 25/25℃均溫型。

M. Rosy Sunset 品種的變方分析結果，顯示日溫 (P = 0.0490)、光強度 (P = 0.0515)、日夜溫度與光強 (P = 0.0165)，以及夜溫與光強間有交感作用存在 (P = 0.0173)，屬於多因子交互影響敏感型品種 (表 4)。受到多因子交互影響，於反應曲面表現上，不同光度環境下之曲面差異極大，當光度為 200 μmol m^{-2} s^{-1} 時，品種對於低夜溫之需求較高，而隨著光度提升，對於夜溫的耐受性亦逐漸提高，但於日溫要求上，仍以 25℃之表現優於高溫 30℃ (圖 3)，整體而言，*M.* Rosy Sunset 之最適生長環境條件為光度 400 μmol m^{-2} s^{-1}，日夜溫環境條件則為 25/25℃均溫型。

表 3. 反應曲面線性模組的變方分析表 (*Colmanara* Massai 'White')。
Table 3. Analysis of variance (ANOVA) for response surface (*Colmanara* Massai 'White').

Source	Sum of squares	*df*	Mean square	*F*	*P*-value Prob > *F*	Result
Model	23.45	10	2.34	6.14	0.0293	significant
A-day	8.02	1	8.02	21.00	0.0059	
B-night	6.20	1	6.20	16.25	0.0100	
C-light	1.896E-003	1	1.896E-003	4.968E-003	0.9465	
AB	1.15	1	1.15	3.00	0.1437	
AC	2.211E-003	1	2.211E-003	5.792E-003	0.9423	
BC	0.56	1	0.56	1.47	0.2793	
A^2	0.12	1	0.12	0.30	0.6053	
B^2	6.03	1	6.03	15.79	0.0106	
C^2	0.050	1	0.050	0.13	0.7310	
ABC	0.42	1	0.42	1.10	0.3419	
Residual	1.91	5	0.38			
Lack of fit	1.81	4	0.45	4.39	0.3418	not significant
Pure error	0.10	1	0.10			
Cor total	25.36	15				

O. Kenneth Biven 'Santa Barbara' 品種的變方分析結果，以夜溫 (P = 0.0348) 與光強度 (P = 0.0035) 為顯著的影響因子，其中光強度影響程度高於夜溫，光強超過 300 μmol m^{-2} s^{-1} 時，光合作用效率明顯提升 (表 5)。於反應曲面圖上，可明確顯示光強度對於光合作用效率的影響，光合作用效率由 200 μmol m^{-2} s^{-1} 起隨光強增強，至 300 μmol m^{-2} s^{-1} 達最高後，隨光強增強而遞減；於溫度表現上，光合作用效率隨溫度升高而下降，相同日溫下，夜溫的上升所造成的光合作用效率降幅大於相同夜溫下日溫提高所造成之影響 (圖 4)。綜合上述結果，推測 *O*. Kenneth Biven 'Santa Barbara' 應適合栽種於中光 300 μmol m^{-2} s^{-1} 左右，日溫 25–28℃，夜溫 20–22℃的環境。

根據本研究之預測結果，推估 *C*. Massai 'White' 的最佳生長條件為日夜溫 25/25℃均溫型、光強度約 200 μmol m^{-2} s^{-1}。*M*. Rosy Sunset 的最佳生長條件為日夜溫 25/25℃均溫型，光強度約400 μmol m^{-2} s^{-1}。*O*. Kenneth Biven 'Santa Barbara' 的最佳生長條件為日夜溫 26/20℃、光強度約 300 μmol m^{-2} s^{-1} (表 6)。

文心蘭盆花品種營養生長實際栽培測試

於 2016 年 4 月至 2017 年 5 月，配合營養生長最佳需求條件測試同步將同品種同規格植株分別放置於新社溫室及古坑網室進行實際栽培測試，試驗期間兩地環境資訊如圖 5 及圖 6。

C. Massai 'White' 於 2016 年 6 月分別置於兩地進行栽培測試，新社溫室植株於 2016 年 11 月達可出貨規格，而古坑網室則延遲至 2017 年 4 月，比較兩地試驗期間環境差異，光度上，新社溫室平均光度可達 181 μmol m^{-2} s^{-1}，古坑網室則因使用遮蔭網降溫，平均光度僅為 72 μmol m^{-2} s^{-1}，正午最高平均光強度亦僅有 160 μmol m^{-2} s^{-1}，於溫度表現上，雖於生長週期平均上兩地差異不大，但透過每月環境變化觀測上可發現新社溫室之日夜均溫均低於古坑網室 (圖 5)，就生長速度上，古坑網室所需時間長達 309 d，為新社溫室的 1.6 倍 (表 7)。該結果與本研究之營養生長最佳需求條件測試結果日夜溫 25/25℃均溫型、光強度約 200 μmol m^{-2} s^{-1} 相符 (表 6)。

M. Rosy Sunset 於 2016 年 4 月開始分別置於新社溫室及古坑網室兩地進行栽培實際測試，

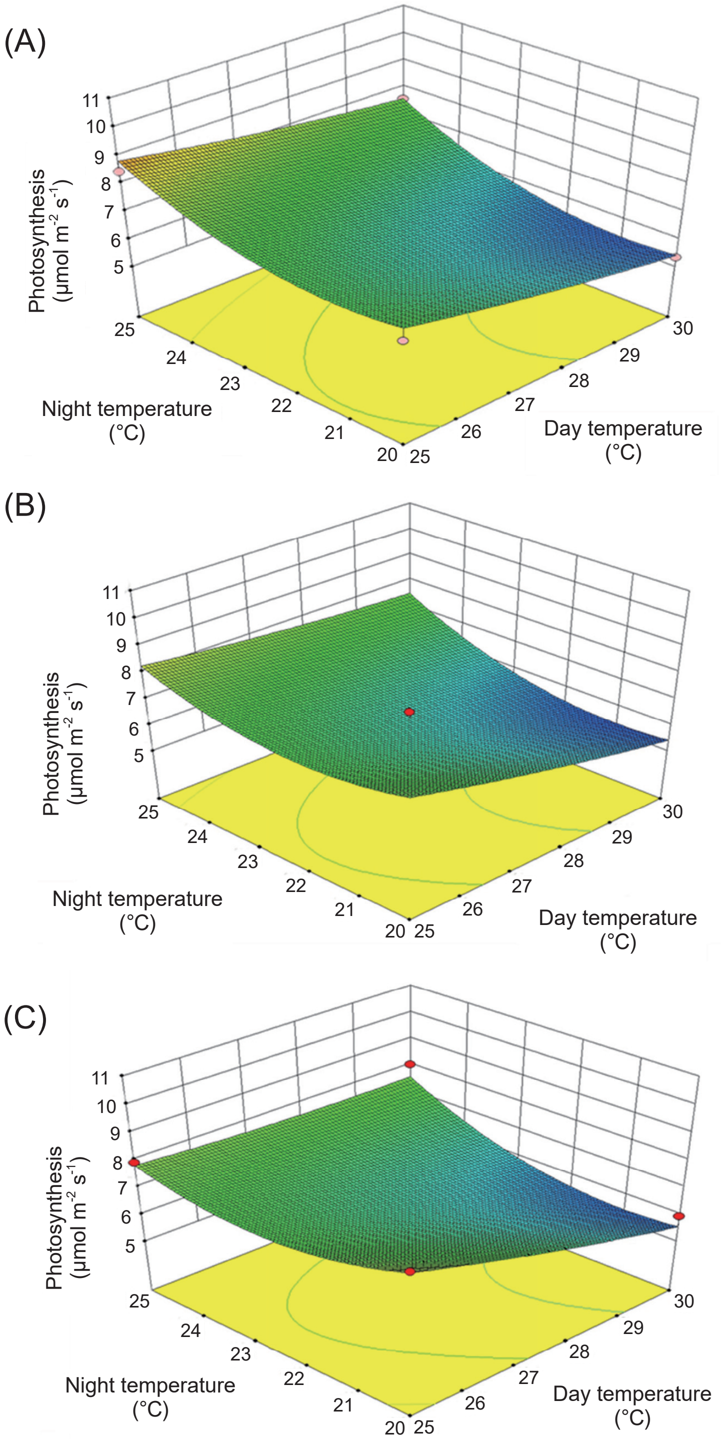

圖 2.　文心蘭盆花生理檢測反應曲面圖，*Colmanara* Massai 'White' (A) 200 μmol m^{-2} s^{-1}、(B) 300 μmol m^{-2} s^{-1} 及 (C) 400 μmol m^{-2} s^{-1}。

Fig. 2.　Response surface plots plotted according to results of potted *Oncidium*, *Colmanara* Massai 'White' (A) 200 μmol m^{-2} s^{-1}; (B) 300 μmol m^{-2} s^{-1}; and (C) 400 μmol m^{-2} s^{-1}.

表 4. 反應曲面線性模組的變方分析表 (*Miltonidium* Rosy Sunset)。
Table 4. Analysis of variance (ANOVA) for response surface (*Miltonidium* Rosy Sunset).

Source	Sum of squares	*df*	Mean square	*F*	*P*-value Prob > *F*	Result
Model	81.00	10	8.10	4.77	0.0249	significant
A-day	9.61	1	9.61	5.66	0.0490	
B-night	5.37	1	5.37	3.16	0.1185	
C-light	9.33	1	9.33	5.50	0.0515	
AB	4.15	1	4.15	2.45	0.1619	
AC	3.18	1	3.18	1.87	0.2136	
BC	16.35	1	16.35	9.63	0.0173	
A^2	4.86	1	4.86	2.86	0.1345	
B^2	3.53	1	3.53	2.08	0.1925	
C^2	13.35	1	13.35	7.86	0.0264	
ABC	16.70	1	16.70	9.83	0.0165	
Residual	11.89	7	1.70			
Lack of fit	10.38	4	2.59	5.16	0.1045	not significant
Pure error	1.51	3	0.50			
Cor total	92.89	17				

兩地生長勢皆於 2016 年 11 月達出貨規格，兩地於試驗期間平均光度均低於200 μmol m^{-2} s^{-1}，僅新社溫室於中午最強光度平均可勉強達近 300 μmol m^{-2} s^{-1}，於溫度表現上，兩地日均溫差異不大，但均高於營養生長最佳需求條件所測得 25℃，然新社溫室因其海拔較高，夜均溫較古坑地區低約 3℃，且整體環境上相較古坑網室較為高光，雖達可出貨規格天數無明顯差異，但於假球莖之周徑上，新社溫室所栽培假球莖周徑可達 89.63 mm，約古坑網室的 1.5 倍 (表 8)，與營養生長最佳需求條件測試結果日夜溫 25/25℃均溫型，光強度約 400 μmol m^{-2} s^{-1} 相符 (表 6)。

O. Kenneth Biven ‘Santa Barbara’ 於2016年 8 月分別置於新社溫室及古坑網室兩地進行栽培，生長勢上分別於 2017 年 4 月與 5 月達出貨規格，因試驗期間適逢台灣冬季，試驗兩地於日夜均溫上差異不大，光度則普遍較低，即便是中午最強光度平均也都低於200 μmol m^{-2} s^{-1}，但新社溫室日間光強度平均為古坑網室的 2 倍，於當代假球莖周徑上尺寸優於古坑網室栽培者，達出貨規格所需天數亦較古坑網室提早約 3 wk (表 9)，而其光強度對於品質上所造成之差異，亦與營養生長最佳需求條件測試結果日夜溫 26/20℃、光強度約 300 μmol m^{-2} s^{-1} 相符 (表 6)。

討論

一般在探討環境因子對植物的生長影響時，多採用複因子試驗 (factorial design) 來進行 (Wallace *et al*. 1984; Rym *et al*. 2010)。本次試驗同時探討日溫、夜溫及光強 3 個變數，加上 5 層級便有 125 個試驗組合，每個試驗條件連續監測 48 h，要將近 8 個多月的時間，即便使用多組生長箱設計，仍無法將試驗時程縮短至符合實際需求，同時隨試驗時間的拉長與試驗植株組數增加，增加了因生長與組間差異所造成的誤差。同時還必須考慮到因子之間的交感作用 (Liyana-Pathirana & Shahidi 2005)，因此複因子試驗非可行方式。利用 RSM 透過有系統的實驗，在各因子介定的範圍內搜尋反應的最適化條件的分析方式，可在 14 d 左右完成 1 個品種的生理特性分析，大幅縮短了檢測時間。利用 DX10 內建的不同的分析模型，不單只可得知進行試驗的日溫、夜溫及光度對品種的影響性外，亦可得知 3 個因子之間的相

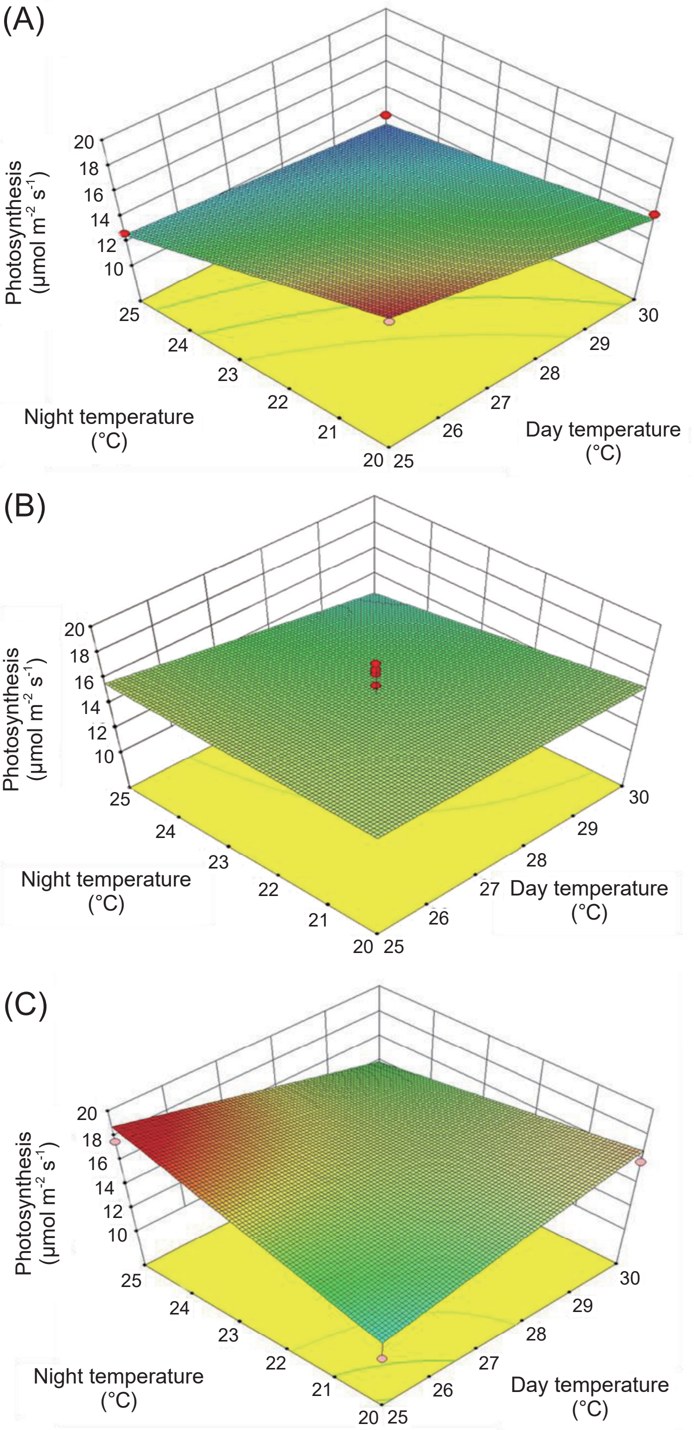

圖 3.　文心蘭盆花生理檢測反應曲面圖，*Miltonidium* Rosy Sunset (A) 200 μmol m^{-2} s^{-1}、(B) 300 μmol m^{-2} s^{-1} 及 (C) 400 μmol m^{-2} s^{-1}。

Fig. 3.　Response surface plots plotted according to results of potted *Oncidium*, *Miltonidium* Rosy Sunset (A) 200 μmol m^{-2} s^{-1}; (B) 300 μmol m^{-2} s^{-1}; and (C) 400 μmol m^{-2} s^{-1}.

表 5. 反應曲面線性模組的變方分析表 (*Odontobrassia* Kenneth Biven ‘Santa Barbara’)。
Table 5. Analysis of variance (ANOVA) for response surface (*Odontobrassia* Kenneth Biven ‘Santa Barbara’).

Source	Sum of squares	*df*	Mean square	*F*	*P*-value Prob > *F*	Result
Model	141.12	10	14.11	5.77	0.0148	significant
A-day	5.72	1	5.72	2.34	0.1699	
B-night	16.69	1	16.69	6.82	0.0348	
C-light	45.75	1	45.75	18.70	0.0035	
AB	4.65	1	4.65	1.90	0.2104	
AC	0.54	1	0.54	0.22	0.6539	
BC	6.94	1	6.94	2.84	0.1360	
A^2	21.72	1	21.72	8.88	0.0205	
B^2	2.91	1	2.91	1.19	0.3118	
C^2	40.85	1	40.85	16.70	0.0047	
ABC	8.39	1	8.39	3.43	0.1064	
Residual	17.12	7	2.45			
Lack of fit	13.53	4	3.38	2.82	0.2103	not significant
Pure error	3.60	3	1.20			
Cor total	158.25	17				

互交感比重作用及是否有交感作用存在，提高對數據的解析能力，從中獲得更多有關該品種的生長特性。

本次試驗的文心蘭品種中，日溫的範圍均在 25–26℃之間，夜溫則可分為 2 群，分別為約 20 ℃的 *O.* Kenneth Biven ‘Santa Barbara’，約 25℃的有 *C.* Massai ‘White’ 與 *M.* Rosy Sunset。顯示試驗的品種多喜歡較冷涼的日夜溫環境，適宜溫度的範圍則依品種而定。光度的部分可分為強光型 *M.* Rosy Sunset (400 μmol m^{-2} s^{-1})、中光型 *O.* Kenneth Biven ‘Santa Barbara’ (300 μmol m^{-2} s^{-1}) 以及低光型 *C.* Massai ‘White’ (200 μmol m^{-2} s^{-1}) (表 6)。

實際栽培測試的調查結果，比較古坑網室與新社溫室兩試驗地之差異，本試驗地點新社溫室海拔約 585 m，於先天環境上，日夜溫均較古坑試驗網室低 (圖 5)，於光度部分，則是新社溫室高於古坑網室，又以 6–8 月之光度差異最為明顯 (圖 6)，古坑網室於夏季時，設施內高溫可達 35℃以上，而高溫會抑制文心蘭光合作用的進行。根據前人研究指出，文心蘭切花品種於台灣 2–6 月以及 9–11 月之光合作用為雙峰類型，即光合作用在午間有午休現象 (midday depression)，而隨著溫度升高，會加劇午休現象的發生；7 月時，當葉溫超過 40℃，淨光合作用甚至會下降接近於零 (Lu 2010)。為了減輕高溫傷害，古坑網室於夏季栽培時，常以遮蔭網等輔助方式降溫，而遮蔭網雖然可阻絕陽光照射降低溫度，但也相對犧牲光度 (圖 6)。光合作用為植物獲取能源的主要途徑，而假球莖為文心蘭的重要貯藏器官，儲存水分、礦物質及碳水化合物 (He 2018)。也因此可見於本試驗中，除了兩地溫度差異影響之外，光度上的明顯落差，使得古坑網室內所栽植之文心蘭假球莖周徑皆不如新社溫室。而於品種生理特性判斷上，於實際栽培上便可見其問題，除了栽培時間冗長 (190–309 d)，更存有更多不穩定之影響因子，如降溫採取措施、病蟲害發生或是劇烈氣候變化等，皆有可能影響最終測試結果。

比較生理檢測系統以及實際栽培測試結果，生理檢測系統可大幅縮短試種時間且比實際種植更能有效掌握受測品種之最適生長條件。檢測結果顯示文心蘭盆花品種 (系) 之生理特性不盡相同，而反應曲面圖所呈現的特性趨勢，可以提供栽培管理調整時的參考依據。

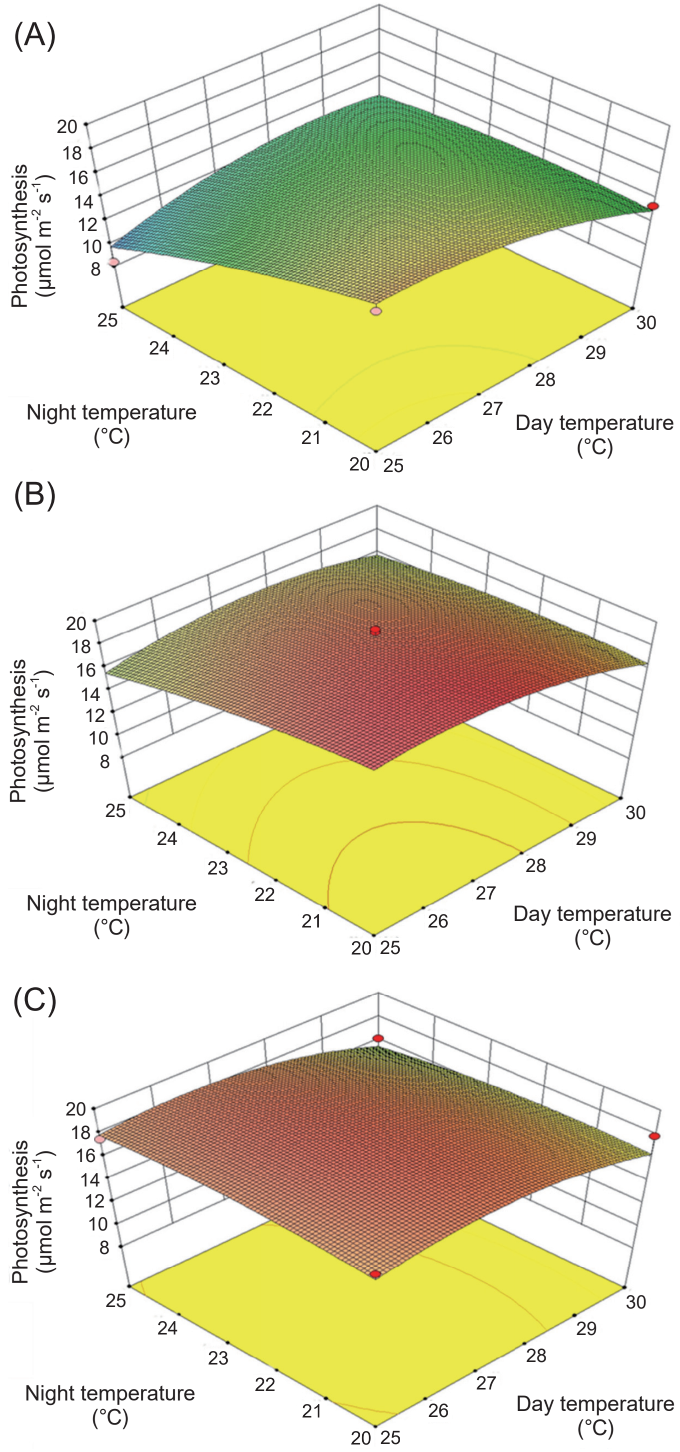

圖 4. 文心蘭盆花生理檢測反應曲面圖，*Odontobrassia* Kenneth Biven 'Santa Barbara' (A) 200 μmol m^{-2} s^{-1}、(B) 300 μmol m^{-2} s^{-1} 及 (C) 400 μmol m^{-2} s^{-1}。

Fig. 4. Response surface plots plotted according to results of potted *Oncidium*, *Odontobrassia* Kenneth Biven 'Santa Barbara' (A) 200 μmol m^{-2} s^{-1}; (B) 300 μmol m^{-2} s^{-1}; and (C) 400 μmol m^{-2} s^{-1}.

表 6.　以反應曲面法推算之文心蘭各品種系最適條件設定值。

Table 6.　The optimum condition of each oncidium variety predicted by the response surface methodology.

Cultivar	Day temperature (℃)	Night temperature (℃)	Light intensity (μmol m^{-2} s^{-1})
Colmanara Massai 'White'	25	25	200
Miltonidium Rosy Sunset	25	25	400
Odontobrassia Kenneth Biven 'Santa Barbara'	26	20	300

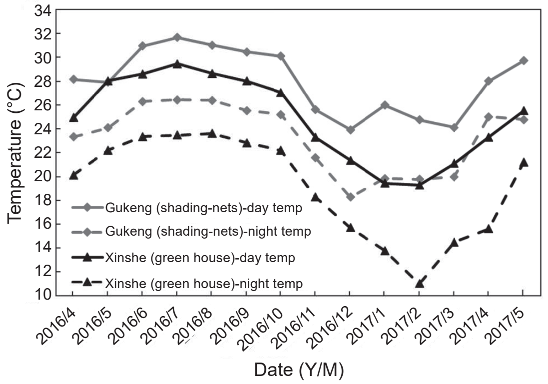

圖 5.　試驗期間測試地區溫度變化。

Fig. 5.　Temperature changes in the test area during the experiment.

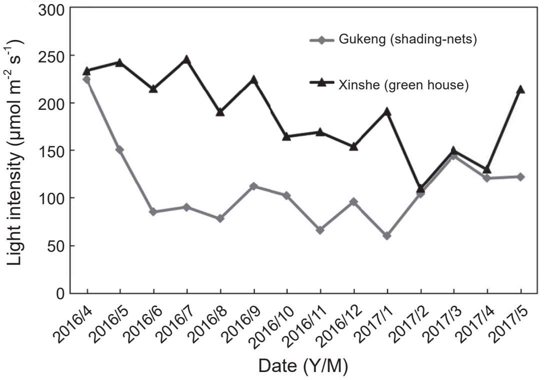

圖 6.　試驗期間測試地區光度變化。

Fig. 6.　Changes in the luminosity of the test area during the experimental period.

表 7.　不同環境下文心蘭盆花品種 *Colmanara* Massai 'White' 實際生長情形。

Table 7.　Actual growth of *Colmanara* Massai 'White' in different environments.

Item	Gukeng shading-nets	Xinshe greenhouse
Day/night temperature (℃)	27.2/21.7	26.7/22.8
Average light intensity (μmol m^{-2} s^{-1})	72	181
Light intensity in the midday (μmol m^{-2} s^{-1})	160	264
Days of reach specification (d)	309	190
Pseudobulb circumference (mm)	88.04	82.76

表 8.　不同環境下文心蘭盆花品種 *Miltonidium* Rosy Sunset 實際生長情形。

Table 8.　Actual growth of *Miltonidium* Rosy Sunset in different environments.

Item	Gukeng shading-nets	Xinshe greenhouse
Day/Night temperature (℃)	29.5/25.1	28.0/22.6
Average light intensity (μmol m^{-2} s^{-1})	119	196
Light intensity in the midday (μmol m^{-2} s^{-1})	186	295
Days of reach specification (d)	196	199
Pseudobulb circumference (mm)	62.16	89.63

表 9. 不同環境下文心蘭盆花品種 *Odontobrassia* Kenneth Biven ‘Santa Barbara’ 實際生長情形。

Table 9. Actual growth of *Odontobrassia* Kenneth Biven ‘Santa Barbara’ in different environments.

Item	Gukeng shading-nets	Xinshe greenhouse
Day/night temperature (℃)	25.5/22.0	24.4/20.7
Average light intensity (μmol m^{-2} s^{-1})	71	153
Light intensity in the midday (μmol m^{-2} s^{-1})	167	173
Days of reach specification (d)	245	266
Pseudobulb circumference (mm)	101.43	112.18

但本試驗是以生長箱模擬控制進行，日夜溫及光環境與實際自然及溫室環境並不完全相同，因此儘管試驗的結果可以推估可能的理想條件設定值，但仍必須持續進行試驗，同時配合業者實際栽培觀察反覆驗證，以累積足夠的檢測經驗及品種特性數據。

透過分析檢測，可在短時間內獲得品種的生理特性，在引種之初就進行評估，減少因現行需實際種植後方能知道該品種適不適合，而造成的時間與成本上的浪費。而就現有的品種而言，可釐清當生長狀況不佳時，是因管理肥培上的問題，還是環境上的問題；依檢測結果進行分群，避免將生長特性差異甚大的品種種植於同一環境中，提高植株良率並降低生產過程中無效的環境控制所產生的費用，進而降低生產成本。並提供後續接力栽培業者在挑選上與種植過程中的參考依據，而達到互利的目標。

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Establishment of Physiological Detection Model for Potted *Oncidium*

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Abstract

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There are many varieties of potted oncidiums, and the diversity of flower shape and color comes from inter-generic and intra-generic hybridization. The differences in their reproductive characteristics often cause cultivation problems in the industry, the method of introducing a small amount of trial seeds is more cost-intensive, and the screening efficiency of suitable varieties is also poor. In this study, a self-designed detection system was used to detect the physiological characteristics of different oncidium varieties and to estimate the optimal environmental conditions for plant cultivation. At the same time, the actual cultivation in different environmental fields was used as a comparison. The test results showed that the tested variety *Colmanara* Massai 'White' was an average temperature type with day and night temperatures of 25/25℃, and the light intensity was about 200 μmol m^{-2} s^{-1}. *Miltonidium* Rosy Sunset was an average temperature type with a day and night temperature of 25/25℃, and the light intensity was about 400 μmol m^{-2} s^{-1}. *Odontobrassia* Kenneth Biven 'Santa Barbara' had a day and night temperature of 26/20℃, and the light intensity was about 300 μmol m^{-2} s^{-1}. The results show that the characteristic trend of the physiological detection system based on the response surface methodology could be used as a reference for the adjustment of cultivation management, and it could also greatly shorten the time for operators to try and explore, and speed up the selection of varieties.

Key words: Response surface methodology, Oncidium, Whole-plant measurement, Photosynthesis.

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利用化學計量分析區分農產品之來源──以茶葉與大蒜為例

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摘要

林振男、彭宗仁、王博軒、林毓雯、劉滄棽。2023。利用化學計量分析區分農產品之來源──以茶葉與大蒜為例。台灣農業研究 72(2):127–149。

本試驗利用化學計量法區分茶葉與大蒜的地理來源。此化學計量分析係利用多變量分析方法，將微量元素含量及同位素組成數據進行統計處理，建立各產地之判別式，並據此估算以判別茶葉及大蒜產地來源。茶葉樣品來自台灣、中國、越南、泰國及印尼；大蒜樣品來自台灣、阿根廷、韓國、中國及越南。分析結果顯示，透過化學計量分析區分茶葉地理來源的整體判別率為 96%，個別地理區判別率最高可達 100%；大蒜試驗的整體判別率為 93%，個別地理區判別率最高可達 100%。除上述優異之判別成果外，有幾個研究經驗可作為後續相關研究之重要參考依據：(1) 微量元素是區分農作物產區地理來源的最重要因子；如果再加入同位素數據，則可提高識別率。(2) 減少參與化學計量分析之地區數目，可提升盲樣判釋的成功率。(3) 應儘量透過相關檢定，篩選出最有相關的自變數以優化判別式。

關鍵詞：化學計量分析、產源判別率、茶葉、大蒜。

前言

在全球化的現況下世界各地皆有頻繁進出口貿易活動。伴隨大眾對食安觀念的提升，消費者選擇購買產品時，價格不再是唯一的影響因素，產品的成分標示及產地來源也愈來愈受到重視 (Kelly *et al*. 2005; Hsu *et al*. 2008)。然因獲利因素，產品的原產地名稱可能被故意竄改。因此，確定各種農產品的原產國，以執行現有的進口法律與法規，並收取適當的關稅，為全球海關部門的職責。基於保護本土農業與消費者權益，具有判斷農產品原產地的能力，對於政府是否能有效實施相關進口法規 (如管理進口配額與特別貿易協定，執行貿易禁運以及徵收反傾銷或反補貼稅等) 甚為重要 (Smith 2005)。

過去 20 年有關追溯農作物來源之研究，多以植體之元素種類及含量特徵做為判定依據 (Macros *et al*. 1998; Fernández-Cáceres *et al*. 2001; Moreda-Piñeiro *et al*. 2001, 2003; Karak & Bhagat 2010)。這是因為植物體的元素多來自於土壤，而土壤的元素特徵主要與母質及施肥歷程有關。因此，經由植體吸收，一地區之土壤母質及環境條件特徵便反映在植體之特定元素含量上。例如，台灣東部由於土壤母質富集鎳及鉻，因此該區植體之鎳及鉻含量，高於台灣其他地區 (Liu *et al*. 2007)；非洲肯亞西部山區的牧草中銅與硒的含量與土壤中的濃度有高度關聯，分析牧草中的銅、硒含量，可分辨各類牧草來源 (Jumba *et al*. 1995)。因此，植物體中的元素含量特徵便可做為追溯農作物來源之依據。

此外，往昔研究顯示可透過分析農產品之氫、氧、碳及氮的穩定同位素組成 (δ^2H、$\delta^{18}O$、$\delta^{13}C$ 及 $\delta^{15}N$) 以確定來源地區 (Rossmann

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2001; Kelly *et al*. 2005; Rodrigues *et al*. 2011; O'Brien 2015)。透過分析作物之 δ^2H 及 $\delta^{18}O$ 以作為地理起源依據的原理，在於水圈中 δ^2H 與 $\delta^{18}O$ 值在空間的變異，具有規律性且可被預測 (Yurtsever & Gat 1981)。簡言之，水圈中 δ^2H 與 $\delta^{18}O$ 值會隨低緯度、低海拔、沿海地區的較高值，向高緯度、山區、內陸地區降低。因作物生長主要利用附近區域水體，因此作物之 δ^2H 及 $\delta^{18}O$ 值與當地的流水，會有很高的相關性，進而作為反映農產品地理來源的判斷依據 (Keppler & Hamilton 2008; Peng *et al*. 2015)。由於各地的氣候環境不同，造成植物會有不同程度的光合作用效率，以致植體間會有不一樣的 $\delta^{13}C$ 值 (O'Leary 1988; Lajtha & Marshall 1994; Hansen *et al*. 2014)。例如生長於較乾旱高溫氣候區的植體，其 $\delta^{13}C$ 值較生長於濕潤較低溫氣候區的植體值還高。因此在不同地點種植的其他相似作物之間其 $\delta^{13}C$ 值的差異，有助於判斷農產品的地理來源。再者，作物從土壤中吸取無機氮，會將其轉為氨基酸。因此，作物的 $\delta^{15}N$ 值與氮源的 $\delta^{15}N$ 值會有很大關係。例如，有機肥料施肥的作物其 $\delta^{15}N$ 值變化範圍為 10–25‰。相比之下，通過合成無機肥料施肥的作物其 $\delta^{15}N$ 值接近大氣 N_2 (0‰)，約為 -5–5‰ (Girard & Hillaire-Marcel 1997; Bateman & Kelly 2007)。由於土壤的 $\delta^{15}N$ 與農民施肥習慣可能因地而異，因此在不同條件下生長的農作物其 $\delta^{15}N$ 可能因地而異，此些差異可能有助於判釋農產品地理來源 (West *et al*. 2009; Zhang *et al*. 2012)。

儘管如此，相較利用單一微量元素含量或同位素組成特徵來解析農產品來源，如果進一步透過化學計量分析 (chemometrics) 將更能達到區別農產品來源的目的 (Santato *et al*. 2012; Liu *et al*. 2014, 2018)。所謂化學計量分析係運用多變量分析方法 (multivariate statistical analysis) 將微量元素含量及同位素組成數據進行統計處理，以區別農產品來源的目的，此統計法主要是找出一群自變數 (元素濃度或同位素組成) 以解釋不同系統 (產地) 間之差異。

由於茶葉為台灣重要經濟作物，台灣製茶技術更是舉世聞名。然而，或聞部分業者進口低價且品質較差之外國茶葉，並偽以台灣產地之茶葉出售，枉顧消費者權益而牟取不當利益。另外，台灣在加入世界貿易組織 (World Trade Organization; WTO) 後，根據政府與 WTO 談判諮商結果，依規定開放大蒜進口，但未開放中國大蒜進口；中國大陸以外地區採關稅配額方式辦理進口。然市場常發生非開放區國家其大蒜來源，卻標示為有配額之國家而進入台灣，造成關稅損失及消費者權益受損。因此，本試驗將以茶葉與大蒜為試驗對象，利用微量元素含量及同位素組成數據，透過化學計量分析，以辨識非台灣產源之農產品。

材料與方法

供試樣品

本試驗供試之茶葉及大蒜樣品為行政院農業委員會農業試驗所於 2014–2018 年收集。茶葉樣品共 199 個，其中台灣樣品 100 個、越南 30 個、泰國 29 個、中國 30 個及印尼 10 個。大蒜樣品共 297 個，其中台灣樣品 213 個、中國 23 個、阿根廷 17 個、越南 27 個及韓國 17 個。此外，為評估本試驗所使用鑑別模式之可利用性，2 個購自馬祖市場的大蒜做為稽核樣品；其中的一個樣品 (標記 VM-C) 自稱來自中國，另一個 (VM-T) 來自台灣。VM-C 及 VM-T 過去也曾做為稽核標本用於主成分分析法之驗證 (Liu *et al*. 2018)。

大蒜及茶葉樣品取回實驗室後進行烘乾及研磨程序。將大蒜樣品蒜瓣分離並剝除大蒜外皮後，置於烘箱去除大蒜植體內的水分，溫度設定為 80℃，持續烘乾 1 wk。烘乾完成後，以鈦刀片植體研磨機，將樣品粉碎研磨成均勻粉末。茶葉樣品放在牛皮紙袋內，放入烘箱以 65℃烘乾 12 h，再利用瑪瑙研缽磨成細粉。研磨完成後的大蒜及茶葉粉末以棕色玻璃瓶存放，並置於防潮箱內保存，提供後續之同位素及微量元素分析用。

穩定同位素組成測定

樣品各穩定同位素組成測定是用先測定樣品的 $^2H/^1H$、$^{18}O/^{16}O$、$^{13}C/^{12}C$ 及 $^{15}N/^{14}N$ 同位素比值，而本試驗每一樣品之同位素比的測定均使用 Thermo Finnigan Delta V Plus 同位素比值

質譜儀 (Isotope Ratio Mass Spectrometry; IRMS) (Thermo Fisher Scientific, Waltham, MA, USA) 進行。該質譜儀以 Con Flo IV 界面連結，其作用在於引入及稀釋工作氣體。測定每個樣品的 $^{2}H/^{1}H$、$^{18}O/^{16}O$、$^{13}C/^{12}C$ 及 $^{15}N/^{14}N$ 後與相對應國際標準品之比值比較，以 δ 表示各同位素的組成，單位為千分比 (‰) (Brand *et al*. 2014)：

$$\delta^{i}E = \delta^{i/j}E = 1{,}000\ [({}^{i/j}R_{S} - {}^{i/j}R_{STD}) / {}^{i/j}R_{STD}] \quad (1)$$

在式 (1) 中 $\delta^{i}E$ 是與氫、氧、碳或氮的各個元素相對應的同位素 δ 值，例如 $\delta^{2}H$、$\delta^{18}O$、$\delta^{13}C$、$\delta^{15}N$ 或 $\delta^{34}S$。${}^{i/j}R_{S}$ 代表樣品的同位素比 ($^{2}H/^{1}H$、$^{18}O/^{16}O$、$^{13}C/^{12}C$ 及 $^{15}N/^{14}N$)，而 ${}^{i/j}R_{STD}$ 是相對應元素的國際標準的同位素比。氫與氧同位素的國際標準品為 Vienna Standard Mean Ocean Water 2 (VSMOW2)；碳同位素為 Vienna Pee Dee Belemnite (VPDB)；氮同位素則為大氣氮 (AIR)。

相關同位素比值的分析細節整理，包括分析時所使用已知 δ 值的標準品，可參閱 Peng *et al*. (2006)。簡言之，樣品粉末藉由元素分析儀 (Elemental Analyzer; EA) (Thermo Finnigan Flash EA 1112, Thermo Fisher Scientific, Waltham, MA, USA) 或高溫轉化元素分析儀 (Thermal Conversion/Elemental Analyzer; TC/EA) (Thermo Fisher Scientific, Waltham, MA, USA) 轉化為相關氣體後經由 Con Flo 界面進入 IRMS。

微量元素測定

植體微量元素之測定方法參考 Lin (2018)。先秤取大蒜或茶葉粉末 0.25 g 放置於消化瓶中，加入 5 mL 的濃硝酸及 0.5 mL 的過氧化氫之消化液，放入微波消化裝置中，加熱至 200℃，維持 10 min。再以 Whatman 42 號濾紙過濾，定量至 100 mL 保存待測。本試驗以感應耦合電漿原子發射光譜分析儀 (Inductively Coupled Plasma-Optical Emission Spectroscopy; ICP-OES) (HORIBA Jobin Yvon ULTIMA 2C, HORIBA, Kyoto, Japan) 測定鐵 (Fe)、錳 (Mn)、鋁 (Al) 磷 (P)、硫 (S)、鉀 (K)、鈣 (Ca) 及鎂 (Mg)，並以感應耦合電漿質譜儀 (Inductively Coupled Plasma-Mass Spectroscopy; ICP-MS) (Agilent 7500 ICP-MS, Agilent, Santa Clara, CA, USA) 測定微量元素，包括硼 (B)、銅 (Cu)、鋅 (Zn)、鎘 (Cd)、鉻 (Cr)、鎳 (Ni)、鉛 (Pb)、砷 (As)、鈦 (Ti)、釩 (V)、鈷 (Co)、鎵 (Ga)、鍺 (Ge)、銣 (Rb)、鍶 (Sr)、鋯 (Zr)、鉬 (Mo)、銀 (Ag)、銫 (Cs)、鋇 (Ba)、鈰 (Ce)、鎢 (W) 及硒 (Se)。

樣品分析同時以空白試驗及標準添加，確認樣品中有無基質干擾或所用的檢測方法是否適當。分析結果以 mg kg^{-1} 表示。至少每 10 個樣品執行 1 個重複樣品分析。若重複分析差異大於 5%，則重複樣品之分析。

化學計量分析

本試驗之化學計量分析乃利用多變量分析方法，將微量元素含量及同位素組成數據進行統計處理。多變量分析又稱為多元統計分析，用於探討多個變數之間的關聯。常用的多變量分析方式，有複迴歸分析 (multiple regression)、因素分析 (factor analysis; FA)、主成分分析 (principal components analysis; PCA)、對應分析 (correspondence analysis; COA) 及判別分析 (discriminant analysis; DA) 等 (Lu 2006)。本試驗先以 COA 及變異係數 (coefficient of variation; CV) 分析移除有可能干擾產地分析之元素變數。最後進行 DA 以建立判別函數 (discriminant function; DF)。

COA 的概念在顯現樣品與變數間的相互關係，幫助瞭解不同樣品與變數之間的關聯。但是 COA 並非敘述變數與樣品之間的因果關係，而僅只是統計學上的對應關係 (Lu 2006)。本試驗利用 COA 檢視元素變數與不同產地來源樣品間的關聯性，並篩選出可作為產地鑑別的重要元素。

CV 為一種相對差異統計量，用以比較單位不同或單位相同但資料差異甚大的資料分散情形，其定義為標準差除以平均值。本試驗利用自變數之 CV 值來瞭解各元素在同一產地、不同樣品間元素的變異程度。如 CV 值過大，表示該元素在同一產地的表現差異過大，不適合用來作為產地鑑別的依據。

DA 之特點為根據一群已分類明確的樣品作為經驗樣品，建立較佳的判別標準或稱判別函數 (DF)，DF 可用來執行分類的工作，當出現未知類別之待判樣品時，可用以判定樣品應歸類於哪一群體中 (Lu 2006)。DA 中利用至少 2 個或 2 個以上自變數產生線性組合，此線性組合需對所定義的群體有最佳的區分能力，而區分能力可透過改變每個變數之權重，使得組間變異達到最大。擁有最大區別能力的線性組合，即為 DA 所需的 DF，其通式如下：

$$DF_a = Z_1X_1 + Z_2X_2 + Z_3X_3 + ... + Z_nX_n + Const \quad (2)$$

其中 DF 為依變數，a 表示樣品產地；Z_n，對自變數 n 的分類權重；X_n，對依變數 DF_a 之第 n 個自變數 (即本試驗中被篩選出的同位素或元素)；Const，常數。

建立各產地之 DF 後，將各樣品之自變數觀測值代入各群所屬的判別函數 DF_a 中，計算樣品在 DF_a 中所得到的數值，在 DF_a 中得到最大值者，就將該樣品歸類於該判別函數所屬之類群 (產地)。依據 DF 對樣品之歸類與已知所屬類群 (產地) 比對，以獲得經 DA 處理的正確性。利用經 DA 被正確歸類的樣品個數除以該群樣品總個數即為判別率。判別率愈高表示該次 DA 處理的可信度愈高。

本試驗之多變量分析使用 2 種統計套裝軟體進行分析，一為 SPSS 14.0 (SPSS Inc., Chicago, IL, USA)；另一為 NCSS 12 (NCSS LLC., Kaysville, UT, USA)。使用這兩種套裝軟體之原因是想比較這兩種商業統計軟體之操作結果是否會有差異。此外，依據本團隊之前的研究經驗，進行多變量分析時，不同自變數 (元素) 數據種類的組合會影響農產品產地來源的判釋 (Tsai *et al*. 2021)。因此，本試驗實驗分析所得之數據的多變量分析，首先是單以茶葉 (或大蒜) 的元素濃度數據組進行，其後再以茶葉 (或大蒜) 的元素濃度數據加上同位素組成數據進行，俾以比較 2 種數據組合之產地鑑別成效差異。

模組驗證

本試驗以 VM-C 及 VM-T 二個大蒜稽核樣品用以驗證前述多變量分析法之適用性。驗證工作以 2 類數據組方式進行：(1) 將 VM-C 及 VM-T 二個樣品之自變數 (元素濃度、同位素組成) 共同與台灣、中國、阿根廷、越南及韓國共 297 個大蒜樣品之自變數做為數據組；(2) 將 VM-C 及 VM-T 二個樣品之自變數只與台灣及中國大蒜樣品之自變數做為數據組。此二類數據組再次進行如前述經 COA、CV 及多變量迴歸分析，並建立台灣及中國之 DF 判別式，再將 VM-C 及 VM-T 二個樣品之自變數，帶入新建立之台灣及中國之 DF 判別式。最後依據 VM-C 及 VM-T 在各產地判別函數 DF_a 中所得數值之高低，驗證 VM-C 及 VM-T 是否確實分屬中國及台灣產地之大蒜標本。

結果

穩定同位素分析

圖 1 為台灣、越南、泰國、中國及印尼各地茶葉 $\delta^{13}C-\delta^{18}O$ 及 $\delta^{13}C-\delta^{15}N$ 的同位素值分布比較圖。由圖 1A 顯示，所有樣品 $\delta^{18}O$ 值主要分布在 12–30‰ 間，而 $\delta^{13}C$ 值則分布在 -22 至 -30‰ 之間，$\delta^{15}N$ 值範圍為 0–10‰ (圖 1B)。

圖 2 為台灣、中國、阿根廷、越南及韓國各地大蒜 $\delta^{2}H-\delta^{18}O$ 及 $\delta^{13}C-\delta^{15}N$ 的同位素值分布比較圖。由圖 2A 顯示阿根廷的 $\delta^{2}H$ 值，明顯低於亞洲地區之值。然而亞洲國家之間，台灣大蒜 $\delta^{2}H-\delta^{18}O$ 值其分布則與中國、越南及韓國有明顯的分區差異。由圖 2B 之 $\delta^{13}C-\delta^{15}N$ 顯示，台灣與阿根廷大蒜的分布區似與其餘地區有區分，但有重疊區域。台灣大蒜的 $\delta^{15}N$ 值較其他國家偏高。

元素分析

本試驗各國茶葉及大蒜樣品之元素平均值及 CV 值，分列於表 1 及表 2；無論是茶葉或大蒜樣品，在所分析的元素組成中，幾乎沒有一種元素的濃度顯著高於其他地區/國家。雖然在茶葉試驗中，印尼茶葉的 Sr 及韓國大蒜的 Zr 平均濃度，較其他地區明顯偏高，但濃度分布範圍會與部分其他國家重疊。除此，任何 2 個區域其任何元素間的濃度差異程度，均低於 10 倍。

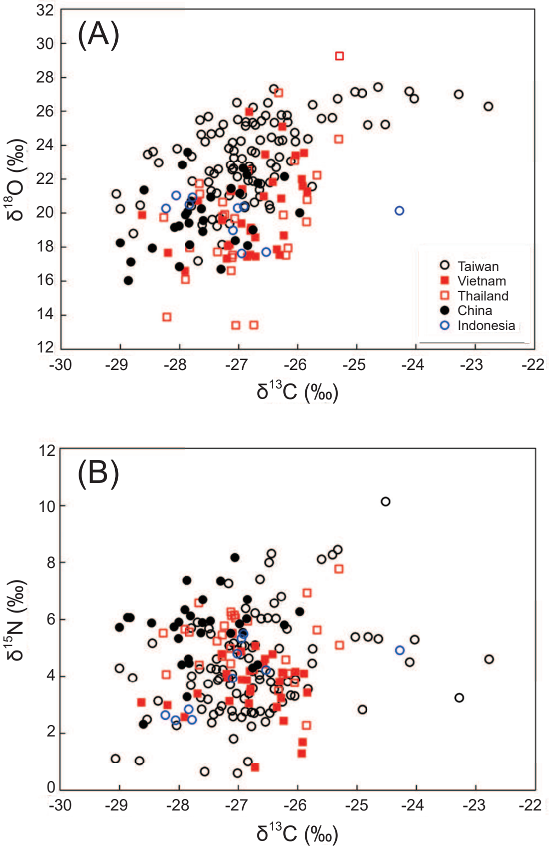

圖 1. 茶葉樣品之同位素散布圖。(A) δ^{18}O vs. δ^{13}C；(B) δ^{15}N vs. δ^{13}C。

Fig. 1. Scatter plots of (A) δ^{18}O vs. δ^{13}C and (B) δ^{15}N vs. δ^{13}C of tea samples.

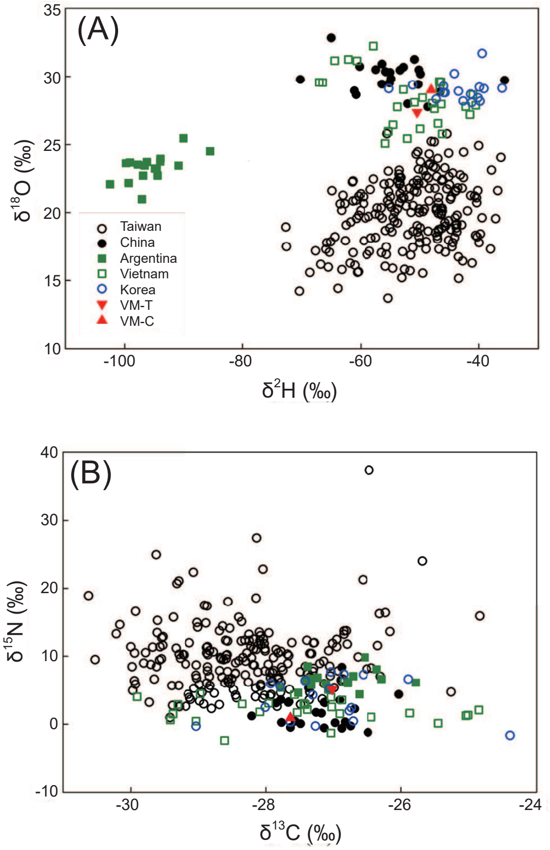

圖 2. 大蒜樣品之同位素散布圖。(A) δ^{18}O vs. δ^{2}H；(B) δ^{15}N vs. δ^{13}C。

Fig. 2. Scatter plots of (A) δ^{18}O vs. δ^{2}H and (B) δ^{15}N vs. δ^{13}C of garlic samples.

由上述元素濃度及同位素分析結果顯示，如同往昔文獻，並無單一茶葉或大蒜之元素濃度，可當作顯著指標以指出農產品來源地區 (Santato *et al*. 2012; Liu *et al*. 2014, 2018)。不同地區之環境條件必然不盡相同，因此植作體內之元素或同位素組成，應該會記錄這些環境條件不同所造成之差異。然而不同地區植作體內之元素或同位素組成差異並非明顯。可能的解釋是不同地區植作體內之元素或同位素組成差異，應是一群組成的成分顯示，組成內的成分會相互影響，單一成分難以顯示其間之差異。因此，利用元素或同位素組成去區分植作的地理來源，應使用 1 組元素成分來解釋差異，而非僅以單一元素予以解釋。

茶葉之化學計量分析

NCSS 分析

首先以 COA 找出產地與變數之間的關聯，並移除無相關變量。由於各元素單位為 mg kg^{-1}，無負值；而穩定同位素組成之單位為千分比 (‰)，大多都為負值。由於 COA 無法處理負值數據，在 COA 處理部分就未將各同位素組成予以列入。

經由 COA 處理產生的雙標圖 (biplot) 可將樣品與變數畫在同一座標軸上，以檢視各樣品與變數間關係。在圖 3 茶葉之雙標圖中，樣品產地以阿拉伯數字表示 (1 = 台灣、2 = 越南、3 = 泰國、4 = 中國、5 = 印尼)，而所輸入之 31 種元素 (變數) 則直接以元素代號標示。在

表 1. 茶葉之元素濃度。

Table 1. Elemental concentrations of tea samples.

Element	Taiwan (n^z = 100)	Vietnam (n = 30)	Thailand (n = 29)	China (n = 30)	Indonesia (n = 10)
B	13.3[y] (22)[x]	15.40 (18)	13.25 (24)	12.69 (16)	16.39 (26)
Cu	8.24 (47)	8.72 (14)	10.06 (16)	6.53 (33)	9.73 (28)
Zn	24.74 (24)	19.81 (18)	24.23 (19)	28.51 (18)	23.44 (12)
Cd	0.038 (44)	0.015 (67)	0.028 (43)	0.03 (40)	0.015 (24)
Cr	0.091 (104)	0.227 (104)	2.748 (92)	0.088 (77)	0.042 (97)
Ni	4.36 (33)	4.115 (35)	4.411 (44)	2.42 (26)	1.717 (62)
Pb	0.245 (44)	0.101 (45)	0.123 (139)	1.301 (70)	0.333 (91)
As	0.028 (50)	0.010 (84)	0.024 (61)	0.030 (45)	0.008 (83)
Ti	1.741 (36)	2.143 (76)	1.352 (43)	1.626 (34)	1.583 (35)
V	0.045 (60)	0.082 (54)	0.052 (60)	0.039 (45)	0.04 (46)
Co	0.259 (104)	0.119 (114)	0.323 (84)	0.160 (61)	0.087 (60)
Ga	0.613 (53)	0.322 (100)	0.900 (59)	0.188 (80)	0.306 (46)
Ge	0.147 (39)	0.159 (38)	0.100 (84)	0.112 (48)	0.099 (50)
Rb	16.261 (39)	26.778 (54)	63.62 (52)	29.84 (31)	39.63 (40)
Sr	9.618 (43)	9.116 (46)	11.69 (48)	15.12 (61)	23.81 (89)
Zr	0.012 (47)	0.028 (101)	0.044 (106)	0.094 (91)	0.053 (129)
Mo	0.019 (125)	0.006 (89)	0.013 (120)	0.025 (219)	0.011 (54)
Ag	0.001 (134)	0.0004 (105)	0.002 (264)	0.004 (88)	0.0005 (113)
Cs	0.061 (115)	0.362 (83)	0.689 (125)	0.166 (42)	0.676 (66)
Ba	6.838 (52)	4.490 (103)	14.18 (68)	2.533 (74)	4.350 (53)
Ce	0.082 (70)	0.085 (94)	0.147 (54)	0.261 (45)	0.056 (25)
W	0.004 (139)	0.001 (164)	0.0009 (174)	0.010 (68)	ND[w]
Se	0.029 (47)	0.095 (54)	0.085 (186)	0.133 (23)	0.044 (38)
P	3,803.8 (19)	3,119.3 (13)	3,250.8 (8)	3,487.1 (12)	3,176.7 (9)
S	2,651 (11)	2,325.4 (11)	2,262.7 (6)	2,639.7 (9)	2,456.7 (7)
K	16,255 (10)	16,433 (8)	15,974 (9)	16,408.8 (10)	1,7306 (9)
Ca	3,468 (17)	3,330.1 (12)	3,781.3 (21)	3,203.6 (16)	3,808.1 (12)
Mg	2,025 (14)	1,930.7 (16)	2,317.1 (13)	2,076.7 (11)	2,031.9 (13)
Fe	71.23 (29)	90.92 (26)	55.87 (21)	86.68 (24)	62.00 (17)
Mn	608.4 (30)	662.74 (53)	912.7 (41)	600.8 (39)	495.0 (35)
Al	486.82 (28)	546.1 (29)	564.9 (28)	637.5 (25)	489.3 (11)
$\delta^{18}O$	23.36 (10)	20.07 (12)	19.78 (18)	19.79 (10)	19.78 (6)
$\delta^{13}C$	-26.74 (-4)	-26.77 (-3)	-26.86 (-3)	-27.60 (-3)	-27.07 (-4)
$\delta^{15}N$	4.25 (42)	3.52 (29)	5.19 (22)	5.65 (21)	3.90 (31)

[z] Number of sample.
[y] Mean, unit in mg kg^{-1}.
[x] Coefficient of variation (CV), unit in %.
[w] Not detected.

表 2. 大蒜之元素濃度。

Table 2. Elemental concentrations of garlic samples.

Element	Argentina (n^z = 17)	China (n = 23)	Vietnam (n = 27)	Korea (n = 17)	Taiwan (n = 213)
B	10.3[y] (40)[x]	6.6 (33)	7.0 (23)	7.0 (14)	8.45 (55)
Cu	4.2 (14)	4.3 (20)	3.9 (33)	2.4 (30)	2.7 (40)
Zn	22.4 (25)	25.1 (36)	28.7 (37)	17.6 (28)	23.6 (54)
Cd	0.034 (46)	0.092 (117)	0.069 (46)	0.055 (28)	0.058 (108)
Cr	0.0142 (170)	0.0379 (423)	0.0078 (425)	0.0251 (309)	0.0411 (237)
Ni	0.13 (30)	0.4 (151)	0.31 (70)	0.19 (146)	0.5 (196)
Pb	ND[w]	4.686 (97)	0.0149 (117)	0.0009 (412)	0.036 (297)
As	0.103 (41)	0.030 (38)	0.02 (62)	0.007 (121)	0.05 (273)
Ti	0.45 (12)	0.48 (51)	0.53 (60)	1.54 (19)	1.285 (77)
V	0.0065 (46)	0.0095 (111)	0.0064 (142)	0.0074 (84)	0.005 (199)
Co	0.0055 (50)	0.0085 (42)	0.0047 (58)	0.007 (78)	0.005 (143)
Ga	0.046 (53)	0.113 (68)	0.070 (67)	0.075 (103)	0.063 (210)
Ge	0.02 (27)	0.006 (100)	0.02 (29)	0.025 (39)	0.034 (64)
Rb	3.4 (83)	4.6 (42)	4.5 (49)	2.7 (43)	1.541 (52)
Sr	5 (19)	3.6 (35)	2 (75)	1.5 (56)	7.927 (216)
Zr	0.0093 (101)	0.1290 (126)	0.0360 (45)	0.41 (15)	0.01 (126)
Mo	0.16 (45)	0.19 (67)	0.097 (51)	0.25 (78)	0.185 (83)
Ag	0.0010 (144)	0.0001 (208)	0.004 (98)	ND	0.004 (631)
Cs	0.0026 (136)	0.0071 (69)	0.0100 (67)	0.0016 (78)	0.001 (100)
Ba	0.5 (54)	0.537 (137)	0.7 (63)	1.1 (99)	0.755 (191)
Ce	0.006 (87)	ND	0.004 (132)	0.001 (88)	0.004 (189)
W	0.0004 (185)	0.0014 (222)	0.0007 (341)	0.003 (75)	0.002 (470)
Se	ND	0.0185 (133)	0.0821 (50)	0.07 (44)	0.048 (120)
P	4,397 (9)	4,315 (16)	4,507 (21)	3,996 (9)	4,137 (19)
S	7,146 (15)	8,702 (26)	7,343 (19)	6,195 (17)	7,212 (21)
K	18,811 (7)	15,614 (11)	15,447 (20)	13,350 (13)	13,148 (49)
Ca	427.3 (12)	498.0 (25)	426.74 (27)	275.8 (41)	1,238 (196)
Mg	768.1 (11)	877.3 (11)	660.9 (22)	685.8 (14)	641.8 (66)
Fe	15.41 (27)	25.56 (40)	35.15 (33)	24.20 (34)	16.4 (70)
Mn	8.00 (12)	10.07 (20)	10.48 (37)	6.96 (36)	6.7 (56)
Al	0.49 (93)	4.70 (151)	83.34 (170)	ND	0.650 (357)
$\delta^{2}H$	-95.53 (-4)	-54.52 (-13)	-51.59 (-15)	-43.45 (-11)	-51.46 (-15)
$\delta^{18}O$	23.31 (4)	29.95 (4)	28.27 (7)	29.03 (3)	19.99 (12)
$\delta^{13}C$	-26.95 (-2)	-27.22 (-2)	-27.44 (-5)	-27.08 (-4)	-28.29 (-4)
$\delta^{15}N$	6.46 (21)	1.87 (124)	2.14 (84)	3.64 (86)	9.72 (50)

[z] Number of sample.
[y] Mean, unit in mg kg^{-1}.
[x] Coefficient of variation (CV), unit in %.
[w] Not detected.

圖 3 中顯示 Cr、Co、Ga、Rb、Mo、Cs 及 Ba 等 7 種元素偏離產地，表示這 7 種元素與產地間關聯低，先予以移除，故剩下 24 種元素。

由於單一方式處理，可能無法完整移除不相關之變數，因此續以剩下之 24 種元素的 CV 值來瞭解各元素在同一產地、不同樣品間元素的變異程度。CV 值愈高的變數代表其在相同樣品來源之差異程度愈大，在解釋產地的能力上並不可靠，因此不適合作為產地鑑別之依據。因為 Zr 在越南、泰國及印尼 3 個產地呈現高度變異性 (CV 值大於 100%) (表 1)，Ag 在台灣、越南、泰國及印尼 4 個產地呈現高度變異性，W 在台灣、越南、泰國及印尼 4 個產地呈現高度變異性，因此，此 3 元素也被視為與產地來源沒有關聯。

在去除上述 Cr、Co、Ga、Rb、Mo、Cs、Ba、Zr、Ag、W 等 10 種變數後，代表剩餘的 21 種元素為低變異性且與產地之間具有較強關聯性，因此本試驗將剩餘之 21 種變數進行判別分析並建立判別函數式。

首先透過逐步迴歸分析將篩選過後的 21 種變數對來自台灣、越南、泰國、中國及印尼共 199 個茶葉樣品之微量元素進行運算，篩選出對依變數有顯著影響的自變數組合。運算結果篩選出 B、Cu、Zn、Cd、Ni、Pb、As、V、Ge、Sr、Ce、Se、S、Mg、Fe 及 Mn 等 16 個變數，並由此 16 個變數建立 5 個具有區分台灣、越南、泰國、中國及印尼茶葉產地來源能力之典型判別函數。接著再對各典型判別函數中之各變數係數值，進行平均數相等性檢定，以得到最佳自變數組合結果。在得到最佳組合結果後，再進一步獲得判別函數之各自變數係數值 (表 3)。依據前述獲得之最適變數及其係數值，建立各茶葉產地來源的判別函數 (DF1N，不含同位素)。以台灣之 DF1N 為例，其展開式如下：

$$
\begin{aligned}
DF1N_T = {} & 1.097B + 0.413Cu + 0.266Zn \\
& - 103.619Cd + 1.251Ni \\
& + 1.304Pb - 166.921As \\
& + 4.674V - 0.331Sr + \\
& 17.381Ce - 11.032Se \\
& + 0.038S + 0.017Mg + \\
& 0.059Fe - 0.001Mn - 79.308
\end{aligned} \qquad (3)
$$

其後，將每個茶葉樣品自變數之觀測值 (元素測值) 分別帶入台灣、越南、泰國、中國及印尼茶葉產地之 DF1N，檢視本試驗茶葉樣品之產地判別率 (表 4A)。結果顯示，在 100 個台灣茶葉樣品中，有 3 個遭到誤判為泰國樣品，2 個誤判為印尼樣品，判別率為 95%；30 個越南樣品中，有 2 個誤判為台灣樣品，1 個誤判為印尼樣品，判別率為 90%；29 個泰國樣品中，有 2 個誤判為台灣樣品，1 個誤判為印尼樣品，判別率為 89.7%；30 個中國樣品，則無誤判，判別率為 100%；10 個印尼樣品中，有 1 個誤判為越南樣品，1 個誤判為台灣樣品，判別率為 80%。DF1N 整體判別率為 93.5 %。

如果將上述在 COA 及 CV 處理後，所篩選出的 21 種元素，再加入 3 種穩定同位素 $\delta^{18}O$、$\delta^{13}C$ 及 $\delta^{15}N$ 以進行 DA 分析處理，進而獲得各茶葉產地來源的判別函數 (DF2N，含同位素)。以台灣之 DF2N 為例，其展開式如下：

$$
\begin{aligned}
DF2N_T = {} & 0.880B - 1.791Cu + 4.663Zn \\
& - 379.095Cd + 6.7481Ni \\
& - 2.335Pb + 1024.238As \\
& - 192.242V - 37.583Ge \\
& - 1.333Sr - 115.636Ce \\
& + 63.964Se + 0.055S + \\
& 0.071Mg - 0.519Fe - \\
& 0.014Mn + 16.894\,\delta^{18}O - \\
& 79.971\delta^{13}C + 6.697\,\delta^{15}N - \\
& 1,463.885
\end{aligned} \qquad (4)
$$

其餘越南、泰國、中國及印尼茶葉產地，如式 (4) 之 DF2N 其最佳自變數組合及各自變數係數值，列於表 3。

其後，將每個茶葉樣品自變數觀測值 (元素及同位素測值) 分別帶入台灣、越南、泰國、中國及印尼茶葉產地之 DF2N，結果顯示 (表 4b)，在 100 個台灣茶葉樣品中，有 1 個遭到誤判為越南樣品，2 個誤判為泰國樣品，1 個誤判為印尼樣品，判別率為 96%；30 個越南樣品中，有 3 個誤判為台灣樣品，1 個誤判為印尼樣品，判別率為 86.7%；29 個泰國樣品中，有 1 個誤判為印尼樣品，判別率為 96.6%；30 個中國樣品均無誤判，判別率為 100%；10 個印尼樣品中，有 1 個誤判為越南樣品，1 個

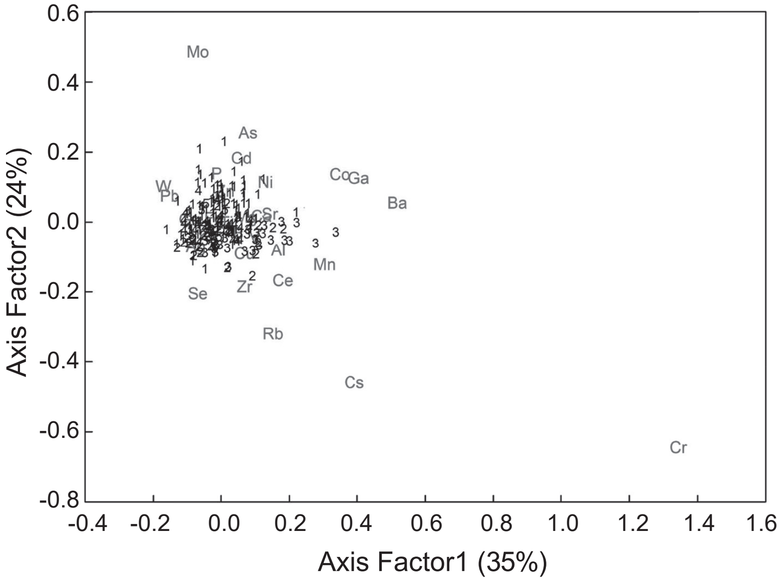

圖 3. 茶葉對應分析之雙標圖，顯示 Cr、Cs、Rb、Ba、Ga、Co 及 Mo 等 7 種元素與本試驗之各產地關聯較小。圖中阿拉伯數字代表產區 (1 = 台灣、2 = 越南、3 = 泰國、4 = 中國、5 = 印尼)，而各自變數直接以元素符號標示。

Fig. 3. Biplots of tea samples produced by correspondence analysis (COA), indicating that the 7 elements of Cr, Cs, Rb, Ba, Ga, Co and Mo have little relationship with geographic origins of this study. In the figure, each sample origins are denoted by Arabic numerals; 1 represents Taiwan; 2, Vietnam; 3, Thailand; 4, China; 5, Indonesia.

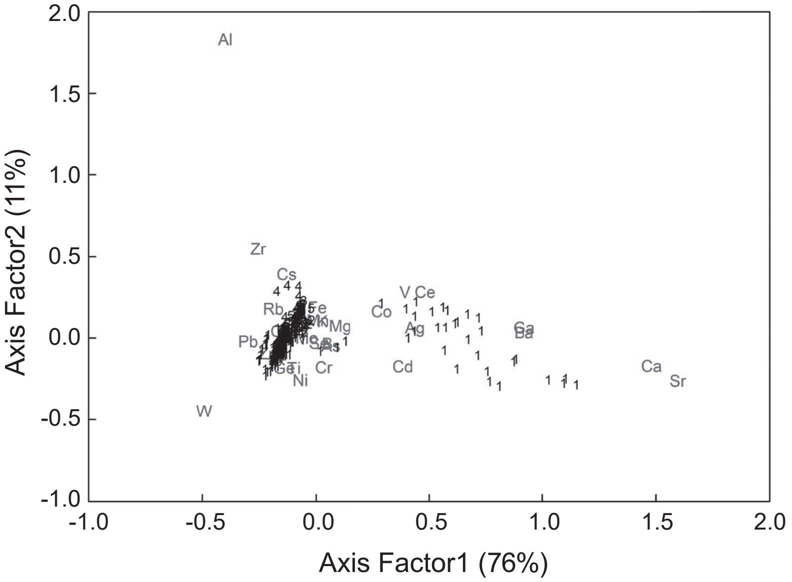

圖 4. 大蒜樣品對應分析之雙標圖，顯示 Al、Sr、Ca、W 及 Zr 等 5 種元素與本試驗之各產地關聯較小。圖中阿拉伯數字代表產區 (1 = 台灣、2 = 中國、3 = 阿根廷、4 = 越南、5 = 韓國)，而各變數直接以元素代號標示。

Fig. 4. Biplots of tea samples produced by COA, indicating that the 5 elements of Al, Sr, Ca, W and Zr have little relationship with geographic origins of this study. Sample origins are denoted by Arabic numerals; 1 represents Taiwan; 2, China; 3, Argentina; 4, Vietnam; 5, Korea. The respective independent variables are denoted by the corresponding elemental symbol.

表 3.　茶葉之判別函數。

Table 3.　Discriminant function (DF) of tea sample of respective geographical origin.

DF[z]	B	Cu	Zn	Cd	Ni	Pb	As	V	Ti	Ga	Ge	Rb	Sr
$DF1N_{T}$	1.097	0.413	0.266	-103.6	1.251	1.304	-166.9	4.674	-[y]	-	-	-	-0.331
$DF1N_{V}$	1.349	0.345	0.114	-221.7	1.730	2.778	-352.7	68.194	-	-	-	-	-0.244
$DF1N_{Th}$	0.822	0.508	0.439	-146.4	0.920	0.874	-162.6	70.873	-	-	-	-	-0.209
$DF1N_{C}$	0.842	-0.078	0.508	-187.3	0.431	10.52	-263.6	-37.59	-	-	-	-	-0.248
$DF1N_{I}$	1.252	0.471	0.256	-231.0	0.250	4.096	-346.3	85.512	-	-	-	-	0.068
$DF2N_{T}$	0.880	-1.791	4.663	-379.1	6.748	-2.335	1024.2	-192.2	-	-	-37.58	-	-1.333
$DF2N_{V}$	1.186	-1.780	4.318	-486.3	6.986	-0.870	808.2	-112.2	-	-	-42.55	-	-1.172
$DF2N_{Th}$	0.692	-1.688	4.753	-424.3	6.345	-2.899	1046.2	-111.2	-	-	-42.94	-	-1.146
$DF2N_{C}$	0.753	-2.263	4.878	-470.2	5.827	6.562	983.3	-217.9	-	-	-64.68	-	-1.180
$DF2N_{I}$	1.095	-1.668	4.529	-495.2	5.519	0.258	840.1	-94.4	-	-	-57.29	-	-0.868
$DF1S_{T}$	1.094	-0.117	0.653	52.511	1.737	-1.845	13.092	-60.01	0.516	30.824	1.836	0.149	-0.309
$DF1S_{V}$	1.108	-0.231	0.533	-99.07	2.528	-0.240	-251.4	43.675	-0.266	8.382	11.700	0.243	-0.245
$DF1S_{Th}$	0.151	-0.065	0.750	-32.12	1.957	-2.111	-121.8	102.80	-3.591	-5.719	42.941	0.487	-0.363
$DF1S_{C}$	0.823	-0.591	0.915	-42.90	1.105	7.048	-114.2	-78.00	0.840	19.193	-20.39	0.197	-0.186
$DF1S_{I}$	1.051	-0.116	0.719	-89.45	1.030	0.620	-229.8	56.102	-0.084	8.807	-2.191	0.239	0.099
$DF2S_{T}$	-0.065	-2.475	4.982	-218.3	5.893	-0.681	1321.7	-435.0	-	55.884	-	0.237	-2.169
$DF2S_{V}$	0.031	-2.516	4.692	-353.3	6.581	0.652	1021.3	-314.2	-	32.686	-	0.317	-2.007
$DF2S_{Th}$	-0.836	-2.367	5.031	-294.0	6.324	-1.148	1244.4	-300.7	-	21.860	-	0.538	-2.198
$DF2S_{C}$	-0.299	-3.000	5.221	-331.2	5.519	8.235	1209.5	-437.4	-	39.535	-	0.284	-2.017
$DF2S_{I}$	-0.077	-2.477	4.939	-355.9	5.149	1.712	1054.4	-304.2	-	31.031	-	0.311	-1.688

表 **3.** 茶葉之判別函數。(續)

Table 3. Discriminant function (DF) of tea sample of respective geographical origin. (continued)

DF[z]	Ba	Ce	Se	S	Ca	Mg	Fe	Mn	$\delta^{18}O$	$\delta^{13}C$	$\delta^{15}N$	Const
DF1N$_{T}$	-	17.381	-11.032	0.038	-	0.017	0.059	-0.001				-79.31
DF1N$_{V}$	-	15.966	3.894	0.036	-	0.017	0.100	0.001				-78.16
DF1N$_{Th}$	-	31.869	13.292	0.030	-	0.023	-0.053	0.004				-76.01
DF1N$_{C}$	-	54.737	25.285	0.037	-	0.020	0.100	0.002				-92.80
DF1N$_{I}$	-	13.309	-5.141	0.039	-	0.017	-0.033	-0.001				-80.49
DF2N$_{T}$	-	-115.64	63.964	0.055	-	0.071	-0.519	-0.014	16.894	-79.97	6.697	-1,463
DF2N$_{V}$	-	-110.97	75.688	0.053	-	0.068	-0.443	-0.012	15.754	-76.66	6.788	-1,343
DF2N$_{Th}$	-	-98.57	88.024	0.048	-	0.075	-0.610	-0.008	16.031	-79.01	7.400	-1,418
DF2N$_{C}$	-	-78.15	101.662	0.055	-	0.072	-0.430	-0.011	16.069	-80.43	8.035	-1,482
DF2N$_{I}$	-	-115.79	67.206	0.057	-	0.069	-0.562	-0.013	15.844	-77.93	6.950	-1,385
DF1S$_{T}$	-2.938	20.409	16.488	-	0.008	0.024	0.216	0.000				-65.59
DF1S$_{V}$	-1.668	20.220	30.107	-	0.010	0.023	0.217	0.002				-71.49
DF1S$_{Th}$	-0.254	30.493	45.600	-	0.010	0.028	0.009	0.007				-84.60
DF1S$_{C}$	-2.542	61.260	50.354	-	0.008	0.027	0.261	0.003				-84.94
DF1S$_{I}$	-1.865	18.745	21.948	-	0.010	0.024	0.119	0.001				-71.09
DF2S$_{T}$	-1.966	-178.1	108.79	-	0.029	-	-0.044	-	14.392	-77.41	8.566	-1,336
DF2S$_{V}$	-0.769	-168.7	117.85	-	0.031	-	-0.016	-	13.264	-74.13	8.465	-1,229
DF2S$_{Th}$	0.484	-166.7	136.83	-	0.032	-	-0.189	-	13.475	-76.82	9.553	-1,319
DF2S$_{C}$	-1.125	-135.8	142.83	-	0.031	-	-0.014	-	13.524	-77.48	9.830	-1,343
DF2S$_{I}$	-0.764	-173.5	111.08	-	0.032	-	-0.136	-	13.312	-75.05	8.609	-1,255

[z] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS. T, Taiwan; V, Vietnam; Th, Thailand; C, China; I, Indonesia.

[y] Not been selected by statistical process.

表 4. 使用表 3 中不同類別之判別函數 (A) DF1N、(B) DF2N、(C) DF1S 及 (D) DF2S 進行茶葉產地來源之判別結果。

Table 4. Discriminating results of tea samples by various types of discriminant function listed in Table 3. Using (A) discriminant function DF1N; (B) discriminant function DF2N; (C) discriminant function DF1S; and (D) discriminant function DF2S.[z]

(A) Actual	Predicted Taiwan	Vietnam	Thailand	China	Indonesia	Sample size
Taiwan	95	0	3	0	2	100
Vietnam	2	27	0	0	1	30
Thailand	2	0	26	0	1	29
China	0	0	0	30	0	30
Indonesia	1	1	0	0	8	10
Discrimination rate	95% (95/100)	90% (27/30)	89.7% (26/29)	100% (30/30)	80% (8/10)	93.5% (186/199)

(B) Actual	Predicted Taiwan	Vietnam	Thailand	China	Indonesia	Sample size
Taiwan	96	1	2	0	1	100
Vietnam	3	26	0	0	1	30
Thailand	0	0	28	0	1	29
China	0	0	0	30	0	30
Indonesia	1	1	0	0	8	10
Discrimination rate	96% (96/100)	86.7% (26/30)	96.6% (28/29)	100% (30/30)	80.0% (8/10)	94.5% (188/199)

(C) Actual	Predicted Taiwan	Vietnam	Thailand	China	Indonesia	Sample size
Taiwan	100	0	0	0	0	100
Vietnam	0	28	1	0	1	30
Thailand	0	0	28	0	1	29
China	0	0	0	30	0	30
Indonesia	1	1	0	0	8	10
Discrimination rate	100% (100/100)	93.3% (28/30)	96.6% (28/29)	100% (30/30)	80.0% (8/10)	97.5% (194/199)

(D) Actual	Predicted Taiwan	Vietnam	Thailand	China	Indonesia	Sample size
Taiwan	100	0	0	0	0	100
Vietnam	1	28	0	0	1	30
Thailand	0	0	29	0	0	29
China	0	0	0	30	0	30
Indonesia	0	1	0	0	9	10
Discrimination rate	100% (100/100)	93.3% (28/30)	100% (29/29)	100% (30/30)	90.0% (9/10)	98.5% (196/199)

[z] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS.

誤判為台灣樣品，判別率為 80%。DF2N 整體判別率為 94.5%。

SPSS 分析

如同上述之 NCSS 處理，首先以 COA 及 CV 之程序，以 SPSS 篩選出只含元素數據之各茶葉產地來源的判別函數 (DF1S)。以台灣之 DF1S 為例，其展開式如下：

$$DF1S_T = 1.094B - 0.117Cu + 0.653Zn + 52.511Cd + 1.737Ni - 1.845Pb + 13.092As + 0.516Ti - 60.005V + 30.824Ga + 1.836Ge + 0.149Rb - 0.309Sr - 2.938Ba + 20.409Ce + 16.488Se + 0.008Ca + 0.024Mg + 0.216Fe - 65.592 \quad (5)$$

其餘越南、泰國、中國及印尼茶葉產地，如式 (5) 之 DF1S 的最佳自變數組合及各自變數的係數值，列於表 3。

其後，將每個茶葉樣品自變數之觀測值 (元素測值)，分別帶入上述各地區之 DF1S 判別式。結果顯示 (表 4C)，100 個台灣茶葉樣品之判別率為 100%；30 個越南樣品中有 2 個誤判，判別率為 93.3%；29 個泰國樣品中，有 1 個誤判為印尼樣品，判別率為 96.6%；30 個中國樣品之判別率為 100%；10 個印尼樣品有 2 個誤判，判別率為 80%。DF1S 整體判別率為 97.5%。

將上述在 COA 及 CV 處理後所篩選出的元素再加入 3 種穩定同位素 $\delta^{18}O$、$\delta^{13}C$ 及 $\delta^{15}N$ 進行 DA 分析處理，可獲得各茶葉產地來源的判別函數 (DF2S)。以台灣之 DF2S 為例，其展開式如下：

$$DF2S_T = -0.065B - 2.475Cu + 4.982Zn - 218.319Cd + 5.893Ni - 0.681Pb + 1321.731As - 434.982V + 55.884Ga + 0.237Rb - 2.169Sr - 1.966Ba - 178.116Ce + 108.791Se + 0.029Ca - 0.044Fe + 14.392\delta^{18}O - 77.413\delta^{13}C + 8.566\delta^{15}N - 1336.081 \quad (6)$$

其餘越南、泰國、中國及印尼茶葉產地，如式 (6) 之 DF2S 的最佳自變數組合及各自變數的係數值，列於表 3。

其後，將每個茶葉樣品自變數之觀測值 (元素及同位素測值) 分別帶入台灣、越南、泰國、中國及印尼茶葉產地之 DF2S 判別式，結果顯示 (表 4D)，100 個台灣茶葉樣品之判別率為 100%；30 個越南樣品中，有 2 個誤判，判別率為 93.3%；29 個泰國樣品判別率為 100%；30 個中國樣品之判別率為 100%；10 個印尼樣品，有 1 個誤判，判別率為 90%。DF2S 整體判別率為 98.5%。

大蒜之化學計量分析

大蒜之統計分析處理程序與上述茶葉相同，以 COA 及 CV 分析移除不適合之自變數變數，再以 DA 分析，以求得各大蒜產地來源的判別函數，並評估其適用性。

NCSS 分析

由大蒜COA分析結果顯示 (圖4)，Al、W、Zr、Ca 及 Sr 等 5 種元素可被移除。由 CV 值分析顯示 Cd、Cr、Ni、Pb、As、V、Ga、Ag、Cs、Ba、Ce 及 Se 等 12 種變數，其 CV 值至少在 2 處產地大於 100% 而被移除。經由 COA 及 CV 處理後，將剩餘之 14 種變數，進行後續之 DA 統計處理後，獲得判別函數各自變數的係數值，建立各大蒜產地來源的判別函數 (DF1N，不含同位素)；以台灣之 DF1N 為例，其展開式如下：

$$DF1N_T = 0.217B + 0.983Cu + 1.716Ti - 87.616Co + 60.865Ge - 0.134Rb + 0.003S + 0.001K - 0.008Mg - 0.113Fe - 0.212Mn - 14.430 \quad (7)$$

其餘中國、阿根廷、越南及韓國大蒜產地，如式 (7) 之 DF1N 的最佳自變數組合及各自變數的係數值，列於表 5。

將本試驗每個大蒜樣品自變數之元素測值，分別帶入上述各地區判別式獲得之判別率，如表 6A 所示。在台灣之 213 個樣品中，有 35 個樣品被誤判，判別率為 83.6%；23 個中國樣

表 **5.** 大蒜之判別函數。

Table 5. Discriminant function (DF) of garlic sample of respective geographical origin.

DF[z]	B	Cu	Zn	Cd	Ti	Co	Ge	Rb	Sr	Se	P
DF1N$_{T}$	0.217	0.983	-[y]	-	1.716	-87.616	60.865	-0.134	-	-	-
DF1N$_{C}$	-0.352	2.399	-	-	0.361	85.422	-114.0	2.113	-	-	-
DF1N$_{A}$	0.119	2.500	-	-	0.076	-81.404	-9.181	1.489	-	-	-
DF1N$_{V}$	-0.079	1.805	-	-	0.378	-137.68	-50.51	1.623	-	-	-
DF1N$_{K}$	-0.032	0.626	-	-	2.024	10.267	21.362	0.806	-	-	-
DF2N$_{T}$	-3.214	9.345	-0.600	-	7.207	1,756.5	-192.6	-3.368	-	-	0.010
DF2N$_{C}$	-4.131	10.410	-0.579	-	6.630	2,068.2	-471.0	-1.096	-	-	0.010
DF2N$_{A}$	-3.511	11.888	-0.796	-	5.612	1,682.3	-288.3	-1.298	-	-	0.014
DF2N$_{V}$	-3.737	9.648	-0.539	-	6.475	1,813.7	-380.1	-1.576	-	-	0.011
DF2N$_{K}$	-3.656	8.084	-0.548	-	8.034	1,966.1	-300.92	-2.404	-	-	0.010
DF1S$_{T}$	0.243	0.861	-	-	1.764	24.022	113.07	-0.223	-0.062	-	0.007
DF1S$_{C}$	-0.252	2.172	-	-	0.465	203.34	-81.36	2.034	0.068	-	0.005
DF1S$_{A}$	0.252	2.195	-	-	0.296	52.880	30.511	1.381	0.650	-	0.004
DF1S$_{V}$	-0.044	1.660	-	-	0.497	-29.62	3.826	1.522	0.361	-	0.006
DF1S$_{K}$	0.045	0.421	-	-	2.126	136.57	67.004	0.712	0.098	-	0.006
DF2S$_{T}$	-3.059	9.433	-0.568	-	7.500	1,727.67	-220.8	-3.517	1.650	-	-
DF2S$_{C}$	-4.011	10.478	-0.529	-	6.896	2,017.7	-509.2	-1.235	1.468	-	-
DF2S$_{A}$	-3.191	11.993	-0.756	-	6.057	1,671.6	-335.1	-1.523	2.334	-	-
DF2S$_{V}$	-3.673	9.755	-0.483	-	6.778	1,734.9	-414.9	-1.733	1.834	-	-
DF2S$_{K}$	-3.564	8.164	-0.498	-	8.301	1,906.7	-335.9	-2.543	1.538	-	-

表 5. 大蒜之判別函數。(續)

Table 5. Discriminant function (DF) of garlic sample of respective geographical origin. (continued)

DF[z]	S	K	Ca	Mg	Fe	Mn	δ^{2}H	δ^{18}O	δ^{13}C	δ^{15}N	Const
DF1N$_{T}$	0.003	0.001	-	-0.008	-0.113	-0.212					-14.43
DF1N$_{C}$	0.003	0.000	-	-0.002	-0.137	0.210					-23.31
DF1N$_{A}$	0.002	0.002	-	-0.015	-0.331	-0.596					-22.46
DF1N$_{V}$	0.002	0.001	-	-0.014	0.201	0.048					-19.49
DF1N$_{K}$	0.002	0.001	-	-0.008	0.045	-0.334					-11.33
DF2N$_{T}$	0.009	-0.004	-	-0.007	-0.342	0.808	-2.663	9.851	-44.31	1.067	-818.3
DF2N$_{C}$	0.010	-0.004	-	0.001	-0.333	1.403	-2.908	13.380	-43.77	0.228	-922.0
DF2N$_{A}$	0.010	-0.003	-	-0.024	-0.602	0.903	-4.091	11.379	-42.42	0.645	-927.4
DF2N$_{V}$	0.009	-0.004	-	-0.011	0.000	1.228	-2.791	12.641	-42.99	0.304	-869.7
DF2N$_{K}$	0.008	-0.004	-	-0.002	-0.161	0.794	-2.429	12.637	-42.93	0.409	-839.0
DF1S$_{T}$	0.001	0.000	0.000	-0.007	-0.204	0.109					-24.28
DF1S$_{C}$	0.002	0.000	-0.002	-0.001	-0.232	0.455					-31.63
DF1S$_{A}$	0.001	0.003	-0.007	-0.019	-0.369	-0.415					-36.75
DF1S$_{V}$	0.001	0.001	-0.003	-0.018	0.166	0.291					-30.54
DF1S$_{K}$	0.001	0.001	-0.002	-0.007	-0.047	-0.043					-21.78
DF2S$_{T}$	0.011	-0.002	-0.015	-0.022	-0.118	0.194	-2.574	8.961	-45.81	1.291	-824.0
DF2S$_{C}$	0.012	-0.002	-0.013	-0.013	-0.102	0.757	-2.772	12.575	-45.17	0.423	-921.3
DF2S$_{A}$	0.012	0.000	-0.022	-0.044	-0.324	0.020	-3.978	9.904	-44.74	0.968	-940.1
DF2S$_{V}$	0.011	-0.001	-0.015	-0.029	0.310	0.483	-2.636	11.843	-44.53	0.543	-870.9
DF2S$_{K}$	0.010	-0.002	-0.013	-0.017	0.090	0.142	-2.292	11.875	-44.32	0.612	-839.1

[z] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS. T, Taiwan; C, China; A, Argentina; V, Vietnam; K, Korea.

[y] Not been selected by statistical process.

表 6. 使用表 5 中不同類別之判別函數 (A) DF1N、(B) DF2N、(C) DF1S 及 (D) DF2S 進行大蒜產地來源之判別結果。

Table 6. Discriminating results of garlic samples by various types of discriminant function listed in Table 5. Using (A) discriminant function DF1N; (B) discriminant function DF2N; (C) discriminant function DF1S; and (D) discriminant function DF2S.[z]

(A)	Predicted					
Actual	Taiwan	China	Argentina	Vietnam	Korea	Sample size
Taiwan	178	2	8	1	24	213
China	0	21	0	1	1	23
Argentina	0	0	17	0	0	17
Vietnam	1	2	0	20	4	27
Korea	1	0	0	0	16	17
Discrimination rate	83.6% (178/213)	91.3% (21/23)	100% (17/17)	74.1% (20/27)	94.1% (16/17)	84.8% (252/297)

(B)	Predicted					
Actual	Taiwan	China	Argentina	Vietnam	Korea	Sample size
Taiwan	212	0	0	0	1	213
China	0	23	0	0	0	23
Argentina	0	0	17	0	0	17
Vietnam	0	1	0	25	1	27
Korea	0	0	0	0	17	17
Discrimination rate	99.5% (212/213)	100% (23/23)	100% (17/17)	92.6% (25/27)	100% (17/17)	99.0% (294/297)

(C)	Predicted					
Actual	Taiwan	China	Argentina	Vietnam	Korea	Sample size
Taiwan	188	1	4	2	18	213
China	0	21	0	1	1	23
Argentina	0	0	17	0	0	17
Vietnam	1	2	0	19	5	27
Korea	0	0	0	0	17	17
Discrimination rate	88.3% (188/213)	91.3% (21/23)	100% (17/17)	70.4% (19/27)	100% (17/17)	88.2% (262/297)

(D)	Predicted					
Actual	Taiwan	China	Argentina	Vietnam	Korea	Sample size
Taiwan	212	0	0	0	1	213
China	0	23	0	0	0	23
Argentina	0	0	17	0	0	17
Vietnam	0	2	0	24	1	27
Korea	0	0	0	1	16	17
Discrimination rate	99.5% (212/213)	100% (23/23)	100% (17/17)	88.9% (24/27)	94.1% (16/17)	98.3% (292/297)

[z] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS.

品中，有 2 個被誤判，判別率為 91.3%；17 個阿根廷樣品之判別率為 100%；27 個越南樣品中，有 7 個被誤判，判別率為 74.1%；17 個韓國樣品中，有 1 個被誤判，判別率為 94.1%。整體判別率為 84.8%。

如果將上述在 COA 及 CV 處理後所篩選出的 14 種元素，再加入 4 種穩定同位素 δ^2H、$\delta^{18}O$、$\delta^{13}C$ 及 $\delta^{15}N$ 進行 DA 分析處理，獲得之產地來源的判別函數 (DF2N，含同位素)；以台灣之 DF2N 為例，其展開式如下：

$$DF2N_T = -3.214B + 9.345Cu - 0.600Zn + 7.207Ti + 1756.548Co - 192.619Ge - 3.368Rb + 0.010P + 0.009S - 0.004K - 0.007Mg - 0.342Fe + 0.808Mn - 2.663\delta^2H + 9.851\delta^{18}O - 44.310\delta^{13}C + 1.067\delta^{15}N - 818.265 \quad (8)$$

其餘中國、阿根廷、越南及韓國大蒜產地，如式 (8) 之 DF2N 的最佳自變數組合及各自變數的係數值，列於表 5。

其後，再以每個大蒜樣品自變數之元素及同位素測值分別帶入上述各地區判別式，獲得之判別率如表 6B 所示。台灣之 213 個樣品中，有 1 個被誤判，判別率為 99.5%；23 個中國樣品之判別率為 100%；17 個阿根廷樣品之判別率為 100%；27 個越南樣品中，有 2 個被誤判，判別率為 92.6%；17 個韓國樣品皆被正確分類，判別率為 100%。DF2N 整體判別率為 99.0%。

SPSS 分析

首先以同樣 COA 及 CV 之處理程序，以 SPSS 篩選出只含元素數據之各大蒜產地來源的判別函數 (DF1S，不含同位素)。以台灣之 DF1S 為例，其展開式如下：

$$DF1S_T = 0.0243B + 0.861Cu + 1.764Ti + 24.022Co + 113.072Ge - 0.223Rb - 0.062Sr + 0.007P + 0.001S - 0.007Mg - 0.204Fe + 0.109Mn - 24.278 \quad (9)$$

其餘中國、阿根廷、越南及韓國大蒜產地，如式 (9) 之 DF1S 的最佳自變數組合及各自變數的係數值，列於表 5。

其後，將每個大蒜樣品自變數之觀測值 (元素測值) 分別帶入上述各地區判別式，結果顯示 (表 6C)，台灣之 213 個樣品中有 25 個被誤判，判別率為 88.3%；23 個中國樣品中，有 2 個被誤判，判別率為 91.3%；17 個阿根廷樣品之判別率為 100%；27 個越南樣品有 8 個被誤判，判別率為 70.4%；17 個韓國樣品之判別率為 100%。DF1S 整體判別率為 88.2%。

進一步，將上述在 COA 及 CV 處理後所篩選出的元素再加入 4 種穩定同位素 δ^2H、$\delta^{18}O$、$\delta^{13}C$ 及 $\delta^{15}N$ 進行 DA 分析處理，獲得大蒜各產地來源的判別函數 (DF2S，含同位素)。以台灣之 DF2S 為例，其展開式如下：

$$DF2S_T = -3.059B + 9.433Cu - 0.568Zn + 7.500Ti + 1727.667Co - 220.789Ge - 3.517Rb + 1.650Sr + 0.011S - 0.002K - 0.015Ca - 0.022Mg - 0.118Fe + 0.194Mn - 2.574\delta^2H + 8.961\delta^{18}O - 45.813\delta^{13}C + 1.291\delta^{15}N - 824.012 \quad (10)$$

其餘中國、阿根廷、越南及韓國大蒜產地，如式 (10) 之 DF2S 的最佳自變數組合及各自變數的係數值，列於表 5。

其後，將每個大蒜樣品自變數之觀測值 (元素及同位素測值) 分別帶入台灣、中國、阿根廷、越南及韓國茶葉產地之 DF2S。結果顯示 (表 6D)，台灣之 213 個樣品中有 1 個被誤判，判別率為 99.5%；23 個中國樣品之判別率為 100%；17 個阿根廷樣品之判別率為 100%；27 個越南樣品有 3 個被誤判，判別率為 88.9%；17 個韓國樣品有 1 個被誤判，判別率為 94.1%。DF2S 整體判別率為 98.3%。

模組驗證

如前述，本試驗之驗證以 2 種方式進行。結果顯示，第一種方式將 VM-C 及 VM-T 兩個大蒜稽核樣品之自變數，與台灣、中國、阿根

廷、越南及韓國共 297 個大蒜樣品之自變數，作為數據組藉由 NCSS 及 SPSS 所建立台灣、中國、阿根廷、越南及韓國大蒜產地各產地之 DF1N、DF2N、DF1S 及 DF2S 中的最佳自變數組合及各自變數的係數值分別與表 5 各產地所列之自變數組合及其係數值相同。其後，將 VM-C 及 VM-T 之自變數數值帶入各產地之 DF1N、DF2N、DF1S 及 DF2S 所獲得之數值，列於表 7A 及表 7B。由表 7A 之 DF1N 得分顯示 VM-C 被誤判為越南標本；由 DF2N (表 7A) 及 DF1S (表 7B) 得分顯示，VM-C 及 VM-T 皆分別被正確歸類為中國及台灣樣品；由 DF2S 得分顯示 (表 7B)，VM-T 被誤判為越南標本。整體而言此類數據組之稽核樣品判別率為 50%。

第二種方式將 VM-C 及 VM-T 稽核樣品之自變數只與台灣及中國大蒜樣品之自變數，作為數據組藉由 NCSS 及 SPSS 所建立台灣與中國產地之 DF1N、DF2N、DF1S 及 DF2S 中的最佳自變數組合及各自變數的係數值如表 8 所示。將 VM-C 及 VM-T 之自變數數值帶入由表 8 建立之台灣與中國產地的 DF1N、DF2N、DF1S 及 DF2S 所獲得之數值列於表 7C。由表 7C 顯示，VM-C 及 VM-T 在所有判別式中皆分別被正確歸類為中國及台灣樣品；此類數據組之稽核樣品判別率為 100%。

討論

判別率與元素組成之關聯

由茶葉判別式 DF1N 與 DF2N (表 3) 顯示，化學計量分析篩選出的相同之主要影響元素，

表 7. 大蒜稽核標本 VM-C 與 VM-T 之判別。(A) 使用表 5 中由 NCSS 建構之判別函數的判別結果；(B) 使用表 5 中由 SPSS 建構之判別函數的判別結果；(C) 使用表 8 中由 NCSS 及 SPSS 建構之台灣及中國判別函數的判別結果。表中粗體數字代表 VM-C 與 VM-T 間分別使用 DF1N、DF2N、DF1S 及 DF2S 所得到之較大值。

Table 7. Discernment of audit garlic of VM-C and VM-T. Discriminating results using discriminant functions based on (A) NCSS listed in Table 5; (B) SPSS listed in Table 5; and (C) NCSS and SPSS of Taiwan and China listed in Table 8. The bold numbers in the table represent the larger values derived by using DF1N, DF2N, DF1S, and DF2S, respectively, between VM-C and VM-T.

(A)	Discrimination function[z]									
Audit garlic	$DF1N_T$	$DF1N_C$	$DF1N_A$	$DF1N_V$	$DF1N_K$	$DF2N_T$	$DF2N_C$	$DF2N_A$	$DF2N_V$	$DF2N_K$
VM-T	**0.914023**	-0.00072	-0.2856	0.548294	-0.0833	**0.365895**	0.277421	0.038496	0.187559	0.13063
VM-C	0.706007	0.060179	-0.33947	**0.742966**	-0.0066	0.171817	**0.346697**	-0.08718	0.320313	0.248356

(B)	Discrimination function[z]									
Audit garlic	$DF1S_T$	$DF1S_C$	$DF1S_A$	$DF1S_V$	$DF1S_K$	$DF2S_T$	$DF2S_C$	$DF2S_A$	$DF2S_V$	$DF2S_K$
VM-T	**20.54987**	18.10877	11.56772	16.59643	17.73894	851.323	857.8406	822.3605	**858.0596**	857.3495
VM-C	13.85233	**16.36621**	5.025302	15.00309	15.47981	852.5964	**872.659**	816.0857	871.6245	872.3165

(C)	Discrimination function[y]							
Audit garlic	$DF1N_T$	$DF1N_C$	$DF2N_T$	$DF2N_C$	$DF1S_T$	$DF1S_C$	$DF2S_T$	$DF2S_C$
VM-T	**3.7730**	-7.3636	**91.3086**	85.4701	**3.0799**	-8.0568	**90.6155**	84.7770
VM-C	3.7107	**4.8570**	109.1413	**130.2735**	3.0176	**4.1639**	108.4482	**129.5804**

[z] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS. T, Taiwan; C, China; A, Argentina; V, Vietnam; K, Korea. Those discriminant functions are constructed by data of all five countries.

[y] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS. T, Taiwan; C, China. Those discriminant functions are constructed by data of Taiwan and China.

表 8. 針對 VM-C 與 VM-T 稽核標本所建立之台灣及中國的判別函數。

Table 8. Discriminant function (DF) of Taiwan and China for audit garlic of VM-C and VM-T.

DF[z]	B	Cu	Pb	Ti	Co	Ga	Ge	Rb	Cs	Mg	$\delta^{18}O$	$\delta^{15}N$	Const
$DF1N_T$	0.243	1.916	0.023	1.328	-[y]	-	43.953	1.068	-482.569	-			-5.805
$DF1N_C$	-0.125	3.731	2.016	0.026	-	-	-89.647	3.734	326.132	-			-21.810
$DF2N_T$	-0.070	1.021	-0.268	-	106.412	20.494	-73.938	0.237	267.977	0.010	4.972	-0.146	-53.238
$DF2N_C$	-0.859	2.634	1.488	-	426.382	35.565	-285.919	2.491	1,292.183	0.019	8.163	-0.933	-149.234
$DF1S_T$	0.243	1.916	0.023	1.328	-	-	43.953	1.068	-482.569	-			-6.498
$DF1S_C$	-0.125	3.731	2.016	0.026	-	-	-89.647	3.734	326.132	-			-22.503
$DF2S_T$	-0.070	1.021	-0.268	-	106.412	20.494	-73.938	0.237	267.977	0.010	4.972	-0.146	-53.931
$DF2S_C$	-0.859	2.634	1.488	-	426.382	35.565	-285.919	2.491	1,292.183	0.019	8.163	-0.933	-149.927

[z] DF1, isotopic data are not involved in statistical process; DF2, isotopic data are involved in statistical process. N, statistical process by NCSS; S, statistical process by SPSS. T, Taiwan; C, China.

[y] Not been selected by statistical process.

有 B、Cu、Zn、Cd、Ni、Pb、As、Sr、Ce、Se 及 Fe，除 Fe 為土壤風化特徵元素外，其餘為土壤之背景微量元素。根據 Tsai *et al*. (2021) 之研究，土壤微量元素及風化特徵元素 Fe、Al 及 Mn 是造成能以 PCA 處理達到區分出台灣茶葉及國外茶葉的主要原因，而肥料特徵元素如 Ca、K、Mg、P 及 S 等，則為 PCA 鑑別之干擾因子。一般而言，微量元素在植物中的含量少，且人為添加的機率低，故可真實地反映茶葉產地之土壤母質特徵。此外，風化程度較高之土壤其 Fe 與 Mn 氧化物含量高。可能因土壤風化程度相異，造成各地植體內 Fe 與 Mn 的含量變異大。再者，依據表 3 列出自變數元素的係數值顯示，屬土壤微量元素之係數值，遠大於風化特徵元素及肥料特徵元素之值。因此，由茶葉判別式 DF1N 與 DF2N 篩選出土壤背景元素為主要自變數之結果，可驗證 Tsai *et al*. (2021) 之研究結果。然而，不同母岩礦物及經歷不同程度的風化作用，都可能造成土壤具相同或相異之風化元素含量特徵，這或許也是在 DF1N 與 DF2N 判別式中，風化特徵元素之係數值不及於微量元素之係數值的原因。

另一方面，由大蒜判別式 DF1N 與 DF2N (表 5) 顯示，化學計量分析篩選出的相同之主要影響元素有 B、Cu、Ti、Co、Ge、Rb、Fe、S、K 及 Mg，與上述茶葉篩選出的元素最大差異，除土壤背景元素及風化特徵元素外，另有肥料特徵元素 S、K 及 Mg 被選入。一般而言，S 與 Mg 為肥料常見元素，由於各地農夫施肥習慣可能不同，因此植體內 S 與 Mg 的差異，可反映產地的不同。再者，如同茶葉一樣，依據表 5 列出自變數元素的係數值顯示，屬土壤微量元素之係數值，遠大於風化特徵元素及肥料特徵元素之值。此結果再次說明土壤微量元素在化學計量分析中，能被用來區別農作物產地差異的最主要因子。

同位素成分之影響

比較茶葉及大蒜判別函數式 DF1N 與 DF2N (表 3)、DF1S 與 DF2S (表 5) 之判別率顯示 (表 4 及表 6)，在 DA 分析中，如果以元素數據組加上 $\delta^{2}H$、$\delta^{18}O$、$\delta^{13}C$ 或 $\delta^{15}N$ 的同位素數據，較單用元素數據，能獲得更佳的判別率，同時

個別地理區判別率達 100% 的數目也有增加，尤以大蒜試驗較為明顯。

例如，在茶葉試驗方面，整體判別率可由單用元素之判別式 DF1N 及 DF1S 的 93.5% 與 97.5% 分別提升到使用元素加上同位素之 DF2N 及 DF2S 的 94.5% 與 98.5% (表 4)；在大蒜試驗方面，DF1N 及 DF1S 之整體判別率分別為 84.8% 及 88.2%，可分別提升到 DF2N 及 DF2S 的 99.0% 及 98.3% (表 6)。此顯示本試驗分析之 δ^2H、$\delta^{18}O$、$\delta^{13}C$ 及 $\delta^{15}N$ 各穩定同位素組成，其環境背景之差異可作為解釋植作地理來源差異的原因。

然而，若直接以同位素數值之差異，作為區別茶業及大蒜產地的解析能力並不佳。例如，茶葉樣品均來自亞洲國家 (台灣、越南、泰國、中國及印尼)，由圖 1B 顯示，各地茶葉的 $\delta^{18}O$ 平均值差異似不明顯，且分布範圍均有重疊。會造成植體中 $\delta^{18}O$ 差異之原因，主要是受各栽種區其區域氣象水文差異所影響，並受植物吸收水分後，其體內同位素分化效應所影響 (Liu *et al*. 2018)。在大蒜試驗中發現，位於南美洲阿根廷大蒜的 δ^2H 值明顯異於任何亞洲國家 (台灣、越南、韓國及中國) 的樣品 (圖 2A)。顯然，δ^2H 值可用於區分洲際間的作物來源，這可能是由於當地的農灌水的 δ^2H 值存在明顯差異造成。然而在亞洲國家間，台灣大蒜樣品的 δ^2H 值與其他越南、韓國及中國樣品之值重疊，但台灣樣品之 $\delta^{18}O$ 值，明顯較其他亞洲國家為低。

另外，圖 1B 顯示各國家茶葉之 $\delta^{15}N$ 值均分布在 0–10‰ 間。一般而言，較高之 $\delta^{15}N$ 值與施用有機肥有關，而較低之 $\delta^{15}N$ 值則與施用化肥有關。在大蒜樣品方面，大多國家樣品之 $\delta^{15}N$ 值均分布在 0–10‰ 間，部分台灣大蒜之值在 10–30‰ 間，意味台灣大蒜栽種施用有機肥情況，較其他國家普遍。另一方面，植體之 $\delta^{13}C$ 值主要與光合作用效率有關 (O'Leary 1988; Farquhar *et al*. 1989)。茶業及大蒜樣品所表現出之 $\delta^{13}C$ 值，為一般典型 C3 植物之值。由圖 1B 及圖 2B 顯示，無論是茶葉及大蒜試驗，無法單由 $\delta^{13}C$ 值去區分產地差異。這表示區域性的栽種條件差異所造成光合作用效率的差異，並不足以判釋各產地間茶葉或大蒜 $\delta^{13}C$ 值的差異。

判別率與樣品數之關聯

判別率的高低似與樣品數有關。在茶葉試驗中 (表 4B)，台灣、越南、泰國及中國各國樣品數在 29 個以上，其判別率為 87–100%；印尼有樣品數 10 個，判別率為 80–90%。然而，此判別率與樣品數之正比關聯，在大蒜試驗方面並不明顯 (表 6)，台灣樣品數 213 個，判別率為 84–99%；阿根廷及韓國樣品數各為 17 個，判別率為 91–100%。因此，判別函數之適用性及判別率的高低與樣品數之關聯須有更進一步的試驗以釋明。

盲樣檢核

本試驗之 NCSS 及 SPSS 兩種統計軟體，都是以多變量分析法所建構而成，雖然在判別分析過程中所篩選出之自變數組合以及各自變數的係數值存在差異，導致判別函數也會不同 (表 3 及表 5)，但最終樣品判別成功率並無明顯差異 (表 4 及表 6)。值得一提的是進行 VM-C 及 VM-T 大蒜樣品之稽核驗證時，在僅選擇中國及台灣之樣品作為分析數據組時，NCSS 及 SPSS 二軟體產生之 2 相對應判別式的自變數種類及其係數值均相同；雖在常數項存有差異 (表 8)，但對稽核樣品判別率均為 100% (表 7C)。因此，NCSS 及 SPSS 二者都是可利用的化學計量分析軟體。

以台灣官方之稽核單位而言，最常面對的問題是 (1) 待測農產品是否為台灣出產？或 (2) 待測農產品其來源地區 (國家) 為何？對於問題 (1) 基本上現階段之化學計量分析技術，以 PCA (Liu *et al*. 2018; Tsai *et al*. 2021) 及本試驗之方法，均可達成。

對於問題 (2)，一般在自我研究中很難做到真正的盲測檢核。本試驗進行盲測檢核的作法，是把待測農產品之自變數帶入各國之判別式，得分最高的即為樣本的來源國。但前提是建立判別式之數據組內，必須有該待測農產品的來源國。但既然是盲測，我們又怎能知道待測農產品的來源國其數據是否被含括在內？以實務而言，任一國家之檢核單位，不可能獲得全世

界各國之標的農作物的元素及同位素數據。

本試驗以 VM-C 及 VM-T，其購自第三地馬祖市場內的 2 個已被告知產地來源的大蒜，作為判別函數的適用性檢驗的稽核樣品，此作法應屬類盲測檢核。如前述，如果把 VM-C 及 VM-T 帶入由台灣、中國、阿根廷、越南及韓國 5 地理區數據組所建立的判別式時，整體判別率僅 50% (表 7A 及 7B)。然而，如果是帶入只用中國及台灣作為分析數據組所建立的判別式時，整體判別率則達 100% (表 7C)。亦即，如果能把可能來源國的數目縮減至最低，則可提升盲樣判釋成功率。至於降低來源國數目能提高判釋成功率的原因，應是進行判別式中的自變數更具地理區分能力。換言之，自變數數量低，代表群組間差異非常明顯；反之，自變數多則代表群組間差異不明顯而不易分辨，因此需較多變數協助分類。以實務而言，在進行驗證工作時，儘量先減少參與化學計量分析之地區 (國家) 數目，其判釋成功率就會愈高。

綜合上述成果，本試驗透過化學計量分析區分茶葉及大蒜之地理來源的整體判別率為 93–96%，個別地理區判別率最高可達 100%。此外，本試驗也獲得幾個重要操作經驗，供後續相關研究參考：(1) 微量元素是區分農作物產區地理來源的最重要因子；如果再加入同位素數據，則可提高識別率；(2) 減少參與化學計量分析之地區數目，可提升判釋成功率；(3) 應儘量透過相關檢定，篩選出最有相關的自變數以優化判別式。

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Apply Chemometrics Approach to Differentiate the Geographical Origin of Agricultural Products: Case Studies of Tea and Garlic

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Abstract

Lin, J. N., T. R. Peng, P. H. Wang, Y. W. Lin, and T. S. Liu. 2023. Apply chemometrics approach to differentiate the geographical origin of agricultural products: Case studies of tea and garlic. J. Taiwan Agric. Res. 72(2):127–149.

This experiment used chemometrics approach to discriminate the geographical origin of tea and garlic. The chemometrics, based on the multivariate analysis, statistically process the trace element concentration and isotopic composition data of agricultural samples studied to establish a discriminant function for each geographical origin. The discriminant function was used to estimate the geographical origin of the tea and garlic studied. The tea samples were taken from Taiwan, China, Vietnam, Thailand, and Indonesia, and the garlic samples were from Taiwan, Argentina, Korea, China, and Vietnam. The results show that the overall discrimination rate of this approach in differentiating the geographical origin of tea was about 96%, and the highest discrimination rate of individual geographical areas was 100%. For comparison, the corresponding discrimination rates of the garlic test were 93% and 100%, respectively. In addition to the excellent discriminant results, some research experiences can be important references for follow-up related research: (1) Trace elements are the most important factor while discriminating the geographical origin of crop production areas; if isotopic data are added, the discrimination rate can be elevated. (2) Minimizing the number of regions involved in the chemometric analysis can elevate the discrimination rate of the blind sample. (3) The most relevant independent variables should be screened out as far as possible to optimize the discriminant function.

Key words: Chemometrics, Discriminant rate of geographical origin, Tea, Garlic.

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研究報告

以光週期進行藍紫色狐狸尾蝴蝶蘭屬 *Rhynchonopsis* Tariflor Blue Kid '1030-4' 花期調節之研究

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摘要

吳容儀、蔡宇玟、戴廷恩。2023。以光週期進行藍紫色狐狸尾蝴蝶蘭屬 *Rhynchonopsis* Tariflor Blue Kid '1030-4' 花期調節之研究。台灣農業研究 72(2):151–160。

藍紫色花為蝴蝶蘭中極罕見之花色，屬內可利用的種原不多，本研究前已透過屬間雜交成功育成 *Rhynchonopsis* Tariflor Blue Kid '1030-4'，藍紫色表現強烈且無褪色情形，其開花習性屬夏開型蘭花，本研究探討光週期對其開花之影響，並期以人為方式調控花期，以達到商業生產所需。研究結果顯示，無論是延長日長或暗期中斷的長日處理均可促進 *R.* Tariflor Blue Kid '1030-4' 提早抽梗與開花，相較於自然日長處理，延長日長處理與暗期中斷處理分別提早 45.5 d 與 31 d 抽梗，兩個長日處理也較自然日長處理的植株分別提早 34 d 與 17 d 開花，延長日長處理對促進植株的抽梗與開花比暗期中斷效果更佳。開花品質方面，延長日長處理的花朵數 (15.6 朵) 顯著高於暗期中斷處理 (8.8 朵) 與對照組 (自然日長) (5.9 朵)，暗期中斷處理的花朵數雖然較對照組多，但兩者並無顯著差異，各處理之花徑並無顯著性差異。花梗長度以延長日長處理的植株最長 (47.2 cm)，其次為暗期中斷處理 (44.7 cm)，而對照組 (自然日長) 之花梗長度為最短 (37.2 cm)；花序長度亦呈現相同趨勢，延長日長處理之花序長度最長 (21.7 cm)，其次為暗期中斷處理 (20.1 cm)，而對照組 (自然日長) 之花序長度則低於其它處理組，為 16.9 cm。各處理之平均單朵花壽命以延長日長處理最長，為 20.1 d，其次為暗期中斷處理 18.2 d，而對照組自然日長之花朵壽命最短，為 17.5 d。

關鍵詞：蘭花、日長、異屬雜交、產期調節。

前言

藍紫色花卉之育成為全世界育種家極力欲達成的目標之一，以蝴蝶蘭而言，目前市場存在的藍色蝴蝶蘭，多數仍是以某些特定的原生種或商業品種雜交或回交而來，蝴蝶蘭屬 (*Phalaenopsis*) 內可利用的種原不多，*Phalaenopsis bellina*、*P. equestris*、*P. javanica*、*P. lowii*、*P. modesta*、*P. violacea* 及 *P. pulcherrima* 等原生種具有藍紫色花之突變種 (Wu *et al*. 2016; Tsao *et al*. 2020)。多數具有藍色花的植物，其花色表現係飛燕草色素 (delphinidin) 累積呈現的結果 (Xu & Dai 2004)，蝴蝶蘭與萬代蘭雖然同屬於指甲蘭亞族 (Aeridinae)，但藍紫色萬代蘭之花色表現，係受到藍色基因調控飛燕草色素之合成，而蝴蝶蘭則缺乏飛燕草色素 (Tatsuzawa *et al*. 1997)。Liang *et al*. (2020) 以高效液相層析儀 (High Performance Liquid Chromatography; HPLC) 分別分析紫色及紫羅蘭－藍花色之蝴蝶蘭，Tsao *et al*. (2020) 分析淺藍紫色與深藍紫色蝴蝶蘭原生種 *P. violacea* fma. *coerulea* 'Taiwan' 與 *P. violacea* fma. *coerulea* 'Norton USA Indigo'，結果皆為以矢車菊素 (cyanidin) 為基底的花

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青素，並未檢測出由藍色基因調控生合成之飛燕草色素；根據主要色素之生合成路徑顯示，矢車菊素為經由紅色素之生合成路徑所形成 (Katsumoto *et al*. 2007)，證實蝴蝶蘭之藍紫色花色表現為受到其他因子影響而呈現出藍紫色，並非由藍色基因調控合成飛燕草色素而來。Wu *et al*. (2022) 透過屬間雜交將藍紫色較強的系統導入蝴蝶蘭中，成功育成藍紫色花的 *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ (Wu *et al*. 2022)，新品種為蘭科蝴蝶蘭屬與狐狸尾蘭屬 (*Rhynchostylis*) 雜交選拔出的藍紫色後代，其開花習性屬夏開型蘭花，自然情況下 1 年開 1 次花，新品系之藍紫花表現強烈且無褪色情形 (Wu *et al*. 2016)。

在商業的生產模式下，新品種必備條件之一，需能以人為調控花期，以俾能計畫生產並提高溫室週轉率，符合產業需求。然而植物的開花誘導 (flower induction) 受到許多如成熟度 (maturity)、溫度、光積值 (daily light integral)、光質 (light quality) 及光週期 (photoperiod) 等因子影響 (Sakanishi *et al*. 1980; Yuan *et al*. 1998; Owen *et al*. 2018; Wang *et al*. 2019)。蝴蝶蘭在營養階段需有充足的養分，才能發育高質量的花，如 *P*. Sogo Lotte ‘F2510’ 與 *P*. Sogo Yukidian ‘V3’ 的花序長度及花朵數，皆隨植株年齡增長而增加 (Tsai & Chang 2022)，因此在營養階段增加光強度會使葉片增厚、葉面積增大，以加速植株的生長，而在生殖階段則可隨光強度增加，以刺激花梗的抽出 (van Tongerlo *et al*. 2021)。在一些蘭花等高等植物上，光週期可以誘導開花 (Wang *et al*. 2003)。*Doritis pulcherrima* (現為 *Phalaenopsis pulcherrima*) 為相對性短日植物 (facultative short-day plants; facultative SDP)，在日/夜溫 30℃/20℃的環境下，9 h 的光週期對花梗創始，比 12 h 更有效率，而且光週期所誘導之開花則與葉片中蛋白質合成的變化彼此密切關係 (Wang *et al*. 2003)。堇花蘭 (*Miltoniopsis* Augres ‘Trinity’) 植株在移到 11–14℃前，於 23℃環境下短日會促進植株開花，但是在涼溫處理期間，不同光週對開花並無顯著影響，此顯示對堇花蘭而言，環境的溫度可能也扮演重要的角色 (Lopez & Runkle 2006)。另一方面，扇形文心蘭 (*Erycina pusilla*) 的花梗形成與延長日長有正相關 (quantitative long-day plant; quantitative LD plant)，顯示扇形文心蘭開花並無一明顯的臨界日長，其對開花的反應屬於相對性長日植物，開花可能視植物體內高碳水化合物累積有關 (Vaz *et al*. 2004)。另外碳氮比 (C/N) 也可作為植株生長及開花潛力的指標，高 C/N 比能促進植株的生殖生長，低 C/N 比則會影響早期的營養生長甚至是抑制開花 (Tsai & Chang 2022)。氮是植物營養及生殖生長重要的營養元素，適當濃度的施用可改善作物生長及開花品質，低濃度的供應會降低生殖階段根及枝條的氮含量，高濃度氮會使 *P*. Sogo Lotte ‘F2510’ 的抽梗延遲 8–9 d，但不會影響開花品質。另外短期施氮的效果與儲存氮庫的大小有關，*P*. Sogo Yukidian ‘V3’ 因儲存大量的氮素，因此較不會影響到開花，但是 *P*. Sogo Lotte ‘F2510’ 則因儲存的氮素有限，所以開花易受影響 (Lin *et al*. 2019)。本研究擬以人為方式調控新品系 *R*. Tariflor Blue Kid ‘1030-4’ 開花期，瞭解其開花習性並期能達到花期調控之目的，以符合商業生產所需。

材料與方法

試驗材料

本研究之參試材料為帶 5–6 片成熟展開葉之 *R*. Tariflor Blue Kid ‘1030-4’ 分生苗，以水苔為栽培介質，種植於 9 cm 盆徑之透明塑膠軟盆。試驗期間每週施以 500 mg L^{-1} 的速效性肥料 Peters 20-10-20 ($N-P_2O_5-K_2O$) 澆灌，其餘時間則視植株之介質表面乾燥情況，以自來水充分澆灌，將介質中累積之鹽類離子淋洗。

試驗方法

試驗期間，植株置於有水牆及風扇設備之溫室中栽培，溫室環境為日/夜溫 26–31℃/18–23℃，溫度低於 18℃則啟動加溫機，光強度在 100–300 μmol m^{-2} s^{-1} 之間 (Chang 2007; Wu *et al*. 2008)，相對濕度為 70–90%。植株施以不同光週期處理：

(1) 自然日長 (natural daylength; ND)：對照組，自然光照約 10.5–11 h。
(2) 暗期中斷 (dark break; DB)：晚上 10:00 至翌日清晨 2:00 點燈進行暗期中斷 (每日 4 h 電照) (Keever *et al*. 2006; Kim 2012; Kim *et al*. 2015)。
(3) 延長日長 (extended daylength; ED)：白天接受自然光照，天黑以後以人工點燈照明，光週期 16 h。

每處理 5 重複，每重複 7 株，共 35 株，本試驗共 105 株參試植株。電照處理之照明設備為長壽特光燈泡 (60W, 110V)，燈泡至參試植株頂端距離約 110–120 cm，照明設備於栽培床架中央之光強度約為 43.9 ± 1.3 Lux。

調查項目

抽梗率 (spiking rate)、雙梗率 (two spikes rate)、抽梗日期 (spiking date)、抽梗所需日數 (days to spiking)、始花日期 (first flower blooming date)、始花出現所需日數、花朵凋謝日期、花朵壽命 (longevity of flower)、花梗長度 (length of stalk)、花序長度 (length of inflorescence)、花朵數 (number of flower)、花朵橫徑 (width of flower) 及花朵縱徑 (length of flower)。

統計分析

試驗設計採完全逢機設計 (complete randomized design; CRD)，以 Costat 6.4 統計軟體 (CoHort Software, Birmingham, UK) 進行變方分析 (analysis of variance; ANOVA) 後，並以最小顯著差異分析 (least significant difference; LSD) 探討各處理間平均值之差異性，另以繪圖軟體 SigmaPlot 10.0 (Systat Software, Chicago, IL, USA) 進行繪圖。

結果與討論

植物在其生命週期中經歷多個發育階段，從萌發開始轉換到幼年營養期 (juvenile vegetative stage)，在這個階段植物對外界環境的誘導信號不敏感，隨後是成年營養期 (adult vegetative phase)，在此階段植物能夠接受外部誘導開花的訊號 (Lopez & Runkle 2006; Ha 2014; Walters *et al*. 2019)。而影響植物開花的原因也不少，許多研究顯示，植物開花受到許多如成熟度 (maturity)、溫度、光積值 (daily light integral)、光質 (light quality) (Kahar 2008) 及光週期 (photoperiod) 等因子影響 (Sakanishi *et al*. 1980; Yuan *et al*. 1998; Kahar 2008; Wien 2014; Owen *et al*. 2018; Han *et al*. 2020)。 蝴蝶蘭與狐狸尾蘭雜交後代 *R*. Tariflor Blue Kid ‘1030-4’ 每年固定於夏天開花，自然情況下 1 年開 1 次花，根據推測其開花受到光週期的影響居多，因此本研究以 *R*. Tariflor Blue Kid ‘1030-4’ 分生苗為試驗材料，以不同光週處理進行人工調節花期試驗。

光週處理對植株抽梗與開花時間之影響

試驗開始後 2 個月，長日處理 (DB，暗期中斷；ED，延長日長) 之植株已有可視花梗出現，此時 DB 與 ED 處理之抽梗率分別為 11.4% 與 8.6%，而在自然日長下之對照組 (ND) 植株則尚未抽梗 (圖 1)。試驗開始後 4 個月，對照組之植株仍未抽梗，而暗期中斷 (DB) 與延長日長 (ED) 處理之植株陸續抽梗，其抽梗率分別為 31.4% 與 51.4% (圖 1)，試驗開始後 5 個月，對照組植株開始有可視花梗出現 (圖 2)，試驗開始後 6 個月，暗期中斷 (DB) 與延長日

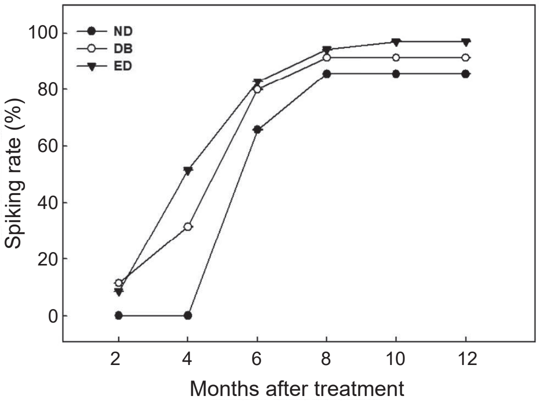

圖 1. 光週處理對 *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ 植株抽梗之影響。ND：自然日長；DB：暗期中斷；ED：延長日長。

Fig. 1. Effects of daylength on stalk emergence of *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’. ND: natural daylength; DB: dark break; ED: extended daylength.

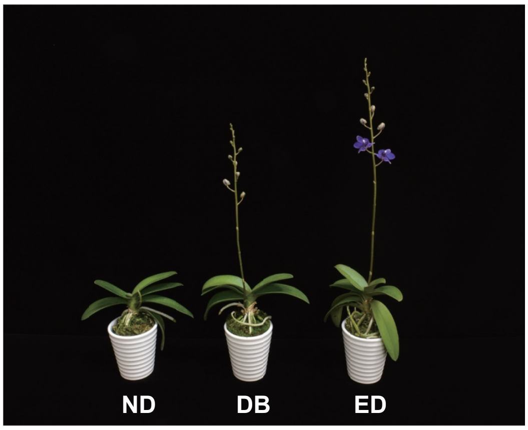

圖 2.　不同光週期對 *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ 植株抽梗開花之影響 (光週處理 5 個月)。ND：自然日長；DB：暗期中斷；ED：延長日長。

Fig. 2.　Effects of daylength on stalk emergence of *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ (5 months after treatment). ND: natural daylength; DB: dark break; ED: extended daylength.

長 (ED) 處理之抽梗率已達 80% 以上 (圖 1)，此時自然日長的植株抽梗率僅 65.7%，處理後 8 個月之暗期中斷與延長日處理的抽梗率已達 90% 以上，延長日長處理的抽梗率接近 100%。試驗開始至花梗出現所需平均日數，以延長日長 (ED) 處理所需時間最短 (113.9 d)，其次為暗期中斷 (DB) 處理 (128.1 d)，而對照組從處理開始至抽梗所需時間最久 (159.1 d)，較延長日長處理約晚 1.5 個月抽梗 (圖 3)。試驗期間，各處理之抽梗率均可達 80% 以上，亦有部分植株出現雙梗情形，以延長日長處理之雙梗率顯著高於其它處理，為 22.7%，而暗期中斷處理與對照組之雙梗率分別為 8.6% 與 5.5%，兩者無顯著性差異 (圖 4)。光週處理後約4.5 個月，延長日長處理之植株已陸續開花，為所有處理中最早開花者，其次為暗期中斷處理，試驗開始後 5 個月，暗期中斷處理之開花率為 2.9% (圖 5)，延長日長處理之開花率為 11.4%，而對照組植株則尚未開花 (圖 5、圖 6)；試驗開始後約 6.5 個月，對照組植株才有第一朵花開放之情形 (193 d)，試驗開始後 7 個月 (圖 5)，延長日長處理之開花率已達 62.9%，為所有處理中開花率最高者，暗期中斷處理次之 (25.7%)，而對照組之開花率最低 (17.1%)；試驗開始後 9 個月，延長日長處理之開花率已達 91.4%，暗期中斷處理次之 (82.9%)，而對

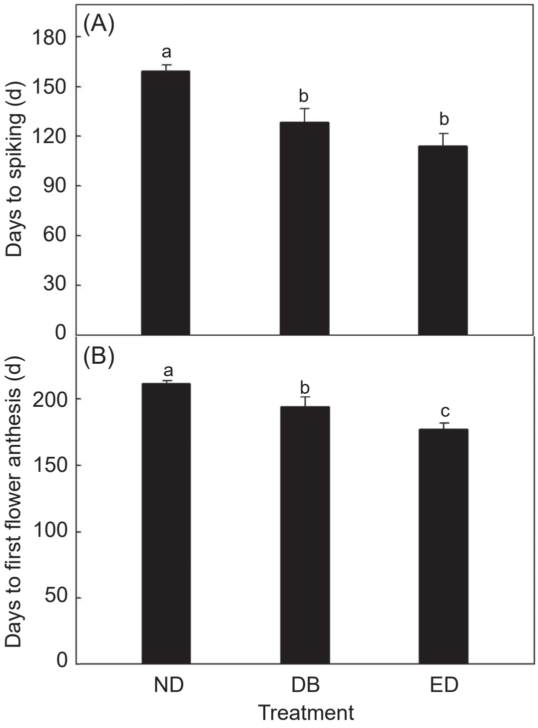

圖 3.　光週處理對 *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ 植株 (A) 抽梗所需日數；(B) 始花出現所需日數之影響。ND：自然日長；DB：暗期中斷；ED：延長日長。

Fig. 3.　Effects of daylength treatment of *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’. (A) Days to spiking; (B) days to first flower anthesis. ND: natural daylength; DB: Dark Break; ED: Extended Daylength.

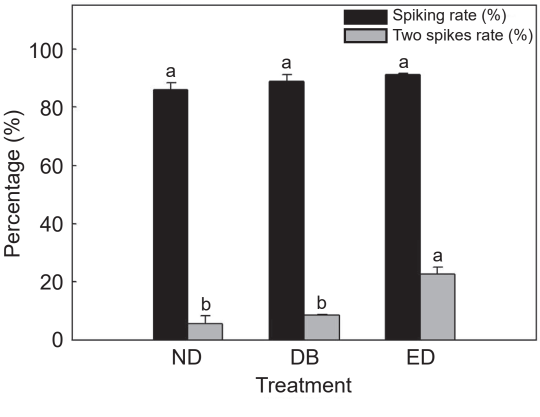

圖 4.　光週處理對 *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ 植株抽梗率與雙梗率之影響。ND：自然日長；DB：暗期中斷；ED：延長日長。

Fig. 4.　Effects of daylength on percentage of stalk emergence and double stalks of *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’. ND: natural daylength; DB: Dark Break; ED: Extended Daylength.

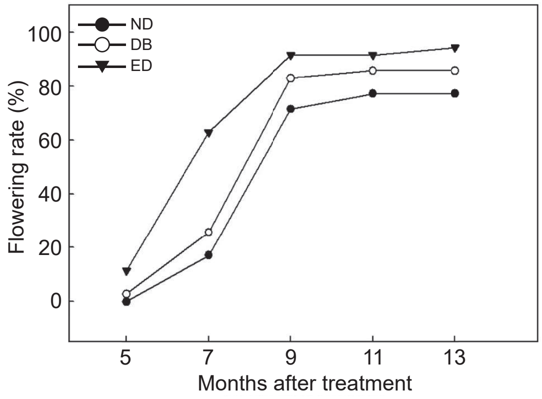

圖 5. 光週處理對 *Rhynchonopsis* Tariflor Blue Kid '1030-4' 植株開花率之影響。ND：自然日長；DB：暗期中斷；ED：延長日長。

Fig. 5. Effects of daylength on percentage of blooming of *Rhynchonopsis* Tariflor Blue Kid '1030-4'. ND: natural daylength; DB: dark break; ED: extended daylength.

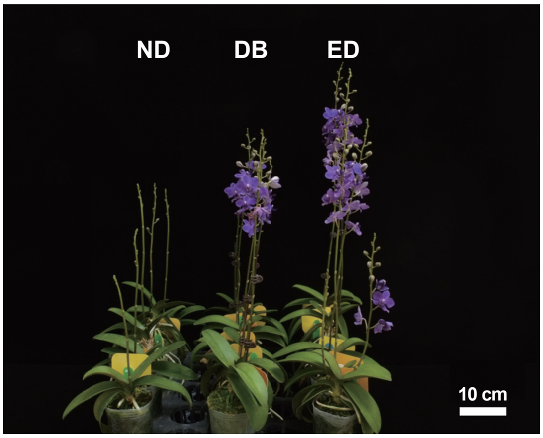

圖 6. 光週處理 6 個月對 *Rhynchonopsis* Tariflor Blue Kid '1030-4' 植株抽梗開花之影響。ND：自然日長；DB：暗期中斷；ED：延長日長。

Fig. 6. Effects of 6-months daylength on percentage of stalk emergence and blooming of *Rhynchonopsis* Tariflor Blue Kid '1030-4'. ND: natural daylength; DB: dark break; ED: extended daylength.

照組之開花率仍為所有處理最低者 (71.4%) (圖 5)。另外，各處理自試驗開始至始花所需平均日數與植株抽梗所需平均日數結果有相同趨勢 (圖 3)，以最早抽梗之延長日長處理，其開花所需時間亦為最短 (177.2 d)，其次為暗期中斷處理 (194.1 d)，而對照組 (自然日長) 之開花所需時間最久 (211.4 d)，亦即對照組植株分別較延長日長處理與暗期中斷處理晚 34 d 與 17 d 開花 (圖 3)。

許多植物開花會受到光週期影響，而植物對光週期的反應則受到光敏素的調節 (Taiz & Zeiger 1991; Craig & Runkle 2016)，光敏素具有 Pr 與 Pfr 兩種型式，其中 Pr 在光照下接收到紅光 (波長 640–690nm) 照射後轉為 Pfr，而 Pfr 在暗期或接收紅外光 (710–750nm) 照射後又轉換成 Pr。根據植物對光週期的不同開花反應，可將之分為 3 大類：短日照植物，需要光週期等於或低於某一個臨界日長；長日照植物，需要高於某一臨界日長植物才能開花；日中性植物，它們的開花不受任何光週期的誘導 (Erwin 2009; Jackson 2009; Torres & Lopez 2011)。而在短日植物與長日植物中，根據其對光週期之非必需 (量的、相對性) 或必需 (質的、絕對性) 反應區分為相對性與絕對性之長短日植物，呈現量反應的物種在任何光週期下都會開花，但在其開花適合之光週期環境下會促進開花；而具有質的反應之植物在獲得所需的光週期之前不會開花 (Ha 2014)。虎頭蘭 (*Cymbidium* 'Red Fire', *Cym.* 'Yokihi')、扇形文心蘭 (*Ercn. pusilla*)、黃鐘花 (*Tecoma stans*)、三色堇 *Viola tricolor* 'Universal Violet'、月見草 *Oenothera biennial* 'Youngii-lapsley' 及洋桔梗 *Eustoma russellianum* 均為長日植物，長光週期會促進植株開花，減少到開花所需天數 (Halevy & Kofranek 1984; Roh *et al*. 1989; Wang *et al*. 2003; Vaz *et al*. 2004; Islam *et al*. 2005; Torres & Lopez 2011; Tuan 2014; Han *et al*. 2020)。洋桔梗 'Echo' 與 'Cessna' 以高壓鈉燈延長日長，其到開花天數比自然日照環境提早 8.5–19.1 d (Paradiso *et al*. 2008)，洋桔梗 'Neil Peach Neo' 以鎢絲燈進行暗期中斷處理 5 h，可見花苞天數較自然日長 (11–12.5 h) 提早 11 d (Yamada *et al*. 2011)。而長壽花 *Kalanchoe glaucescens*、*K. manginii*、*K. uniflora*、*K. laciniata*、*K. millotii*、*K. nyikae*、*K. rotundifolia* 及 *K. velutina* 則為絕對性短日植物 (Currey & Erwin 2010, 2011)。本試驗結果 (圖 2、圖 3、圖 4) 顯示，無論是延長日長或暗期中斷的長日處理，均可促進 *R*. Tariflor Blue Kid '1030-4' 植株提早抽梗與開花，相較於自然日長處理，延長日長處理與暗期中斷處理分別提早 45.5 d 與 31 d 抽梗，兩個長日處理也

較自然日長處理的植株分別提早 34 與 17 d 開花，延長日長處理對促進植株的抽梗與開花又比暗期中斷效果更佳。Jackson (2009)、Torres & Lopez (2011)、Tuan (2014) 提出，當植物處於臨界日長或以上的環境時，才能獲得開花能力者為長日植物。由試驗結果可知，*R*. Tariflor Blue Kid ‘1030-4’ 植株開花對光週期的反應，在長日環境下生長的植株抽梗與開花受到促進，可提早開花，因此歸類為長日植物。而許多文獻也提到，在長日與短日植物中，呈現量反應的植物在任何光週期下都會開花，這是一種相對性光週反應，即當植物處於特定臨界日長以上或以下可以加速開花；而具有質反應的植物則必須生長於特定臨界日長才會開花，此為絕對性光週反應 (Vaz *et al*. 2004; Torres & Lopez 2011; Ha 2014)。*R*. Tariflor Blue Kid ‘1030-4’ 於自然日長環境中生長的植株，無論抽梗或開花雖然較兩個長日處理需要比較長的時間，但最終植株仍然開花，可見長日對其有促進開花之效。

光週處理對植株開花品質與其它性狀之影響

植物開花會受到光週期影響之種類，除了光週期長度會影響開花能力與開花時間 (Kahar 2008; Currey & Erwin 2010, 2011; Wien 2014)，也會影響其它特性之表現，但是各性狀表現則隨植物種類或品種而異 (Kahar 2008; Torres & Lopez 2011; Wien 2014)。本試驗開始後 8 個月調查各處理之開花品質，結果顯示，以延長日長處理的花朵數 (15.6 朵) 顯著高於暗期中斷處理 (8.8 朵) 與對照組 (自然日長) (5.9 朵)，暗期中斷處理的花朵數雖然較對照組多，但兩者並無顯著差異，而各處理之花徑並無顯著性差異 (表 1)。當各處理之花朵開放數量達 7 朵時，調查其花梗與花序長度，結果顯示，延長日長處理的花梗長度最長 (47.2 cm) (表 1)，其次為暗期中斷處理 (44.7 cm)，而對照組 (自然日長) 之花梗長度為最短 (37.2 cm)；延長日長處理之花序長度最長 (21.7 cm)，其次為暗期中斷處理 (20.1 cm)，而對照組 (自然日長) 之花序長度則低於其它處理組，為 16.9 cm (表 1)。各處理之平均單朵花壽命以延長日長處理最長，為 20.13 d，其次為暗期中斷處理 (18.19 d)，而對照組自然日長之花朵壽命最短，為 17.46 d (表 1)。本試驗所使用之燈泡屬於白熾鎢絲燈，其紅光/遠紅光比例低 (Abdel-Rahman *et al*. 2017)，當長日植物黃鐘花 (*T. stans* ‘Mayan Gold’) 在低紅光/遠紅光比例的光照環境下生長時，莖伸長會受到促進 (Torres & Lopez 2011)，再者，黃鐘花在長日下 (日長 14 d 或更長) 生長時，花朵數與分枝數增加，而在短日照下 (12 h 或更短)，很少有植株出現可視花芽；短日植物菊花 (*Chrysanthemum morifolium* cv. Reagan Sunny) 在花芽創始與花芽發育期，植株生長不受光週期的明顯影響，8 h 的光週期會促進開花，但花朵品質會受到光週期的影響，在花芽創始期短日處理 (8 h) 會導致花序、花徑與觀賞壽命顯著減少，花梗長度上亦有類似趨勢，但差異不顯著，苞片數量則呈現相反的趨勢，8 h 時略有增加，10 h 時顯著增加 (Kahar 2008)；向日葵 (*Helianthus annuus* cv. Sunrich Orange) 在短日下 (12 h) 會提早開花，然而植株高度僅有長日下植株 59% 的高度，花朵明顯更小，葉片數也減少 (Wien 2014)。Halevy & Kofranek (1984) 也曾提出洋桔梗的花朵數不會受到光週期影響。洋桔梗 ‘Echo’ 與 ‘Cessna’ 於補充 200

表 1. 光週處理對 *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’ 開花品質之影響。
Table 1. Effects of daylength on flower quility of *Rhynchonopsis* Tariflor Blue Kid ‘1030-4’.

Daylength	Number of flower	Flower width (cm)	Flower length (cm)	Flower width/ flower length	Longevity of flower (d)	Length of stalk (cm)	Length of inflorescence (cm)
Control (natural daylength)	5.90 b	3.58 a	3.12 a	1.15 a	17.46 b	37.2 b	16.9 b
DB (dark break)	8.80 b	3.56 a	3.14 a	1.14 a	18.19 ab	44.7 ab	20.1 a
ED (extended daylength)	15.60 a	3.57 a	3.12 a	1.15 a	20.13 a	47.2 a	21.7 a

μmol m^{-2} s^{-1} 高壓鈉燈以延長日長為 18 h 處理之花朵數，與自然日照環境亦無顯著差異 (Paradiso *et al*. 2008)。本試驗在延長日長環境下，*R*. Tariflor Blue Kid ‘1030-4’ 的花朵數顯著較其他 2 個處理來得高，在花梗長度、花序長度與花朵壽命也稍受到光週期影響表現，花徑則未受到光週期影響，本試驗長日處理的花梗與花序較長，可歸因於植株處於低比例紅光/遠紅光環境比較長的時間所致。由上所述，可見光週期對植物各性狀表現會隨種類或品種而有所不同 (Kahar 2008; Torres & Lopez 2011; Wien 2014)。

結論

有關植物的花芽分化與開花機制，正確來說，應是暗期時間的長短所控制的發育行為，也就是每天的連續黑暗時數會誘導植物的形態發生轉變，然目前仍多沿用舊稱以光週期 (而非暗週期) 表示 (Taiz & Zeiger 1991; Lin & Yang 2011)。近年來，花卉市場為提供消費者多樣化產品之選擇，許多育種者也嘗試跨屬間之雜交，以期創造令人耳目一新的花卉形態與花色。然而要成為商業市場所接受的產品，花期調控技術需被建立，本篇研究已初步建立蝴蝶蘭與異屬間雜交種之花期調節技術，未來將持續致力於相關花期調節機制研究，期能應用於實務生產上，提升台灣花卉產業競爭力。

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Study on Flowering Regulation of *Rhynchonopsis* Tariflor Blue Kid '1030-4' by Photoperiodism

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Abstract

Wu, J. Y., Y. W. Tsai., and T. E. Dai. 2023. Study on flowering regulation of *Rhynchonopsis* Tariflor Blue Kid '1030-4' by photoperiodism. J. Taiwan Agric. Res. 72(2):151–160.

Indigo Phalaenopsis is rarely seen in the commercial market and just a few germplasm are available in the genus for indigo flower breeding. *Rhynchonopsis* Tariflor Blue Kid '1030-4' with a deep and nonfading blue color has been bred successfully by intergenic hybridization. The purpose of this study is to discuss the effect of photoperiod on the flowering of *Rhnps*. Tariflor Blue Kid '1030-4', and plan to regulate artificially the flowering period for commercial production. The results of the study showed that long-day treatments including extending day length (ED) and dark break (DB) could promote the early stalk emergence and flowering of *Rhnps*. Tariflor Blue Kid '1030-4'. Stalk emergence of plants treated with ED and DB were 45.5 d and 31 d earlier than natural day length (ND) treatment, respectively, and flowering time that was 34 d and 17 d earlier than control, respectively. ED has a better effect than DB on promoting stalk emergence and flowering. The number of flowers in the ED treatment (15.6 flowers) was significantly higher than that in the DB treatment (8.8 flowers) and control (ND) (5.9 flowers), however, there was no significant difference between DB and control. There was no significant difference in the flower diameter among the three treatments. The longest stalk (47.2 cm) in that of ED, followed by DB treatment (44.7 cm), while the shortest stalk of the control group (ND) was 37.2 cm. The inflorescence length also showed the same trend. The longest inflorescence (21.7 cm) in that of ED, followed by DB treatment (20.1 cm), while the shortest inflorescence appeared in the control (16.9 cm). The longest longevity of a single flower in ED was 20.1 d, followed by DB treatment (18.2 d), while the shortest longevity was 17.5 d.

Key words: Orchid, Daylength, Intergenus-hybridization, Flowering regulation.

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研究報告

以 GGE 雙軸圖評估水稻糙米鎘濃度之品種變異性及其環境交感效應

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摘要

許健輝、林毓雯、楊博鈞、莊淞麟、鄭佳怡、莊愷瑋。2023。以 GGE 雙軸圖評估水稻糙米鎘濃度之品種變異性及其環境交感效應。台灣農業研究 72(2):161–179。

水稻為亞洲國家的主食，水田土壤受鎘污染導致水稻穀粒的鎘累積，使鎘米事件的議題備受關注，水稻穀粒累積鎘的差異性不僅與環境與品種有關，也受品種與環境交感效應所影響。本研究應用 GGE 雙軸圖解析影響穀粒鎘累積之品種效應 (G) 以及品種與環境的交感效應 (G × E)，以主成份 (principle components) 得分繪圖說明品種間差異受環境影響的程度；本研究分別於台中市后里區 (Houli; HL) 及彰化縣彰化市 (Changhua; CH) 兩處土壤鎘污染之試驗田區，栽種國內常見的 21 種水稻栽培種，經試驗調查取得土壤與水稻穀粒樣本，分析測定獲得不同品種水稻根圈土壤及糙米鎘濃度之資料，供 GGE 雙軸圖分析以篩選出環境穩定的低鎘累積品種。研究結果顯示，秈型稻的鎘累積能力於兩處試驗區皆呈現高於稉型稻的趨勢；因兩處試驗區 HL 與 CH 的環境差異對不同品種的影響程度不同，兩處試驗區糙米鎘濃度較低之品種並不一致。再者，經 GGE 分析可將品種及品種與環境交感效應的主導變異轉換至 2 項獨立主成份 (即是 PC1 與 PC2) 可達 84%，經繪製雙軸圖有效呈現出不同品種水稻糙米鎘濃度對應兩處試驗區環境效應的差異性，其結果顯示「高雄 139 號」(‘KH139’)、「台稉糯 3 號」(‘TKW3’)、「台農糯 73 號」(‘TNGW73’) 及「台東 33 號」(‘TT33’) 相較其他品種，兼具環境穩定且低鎘累積之特性。因此，試驗區土壤鎘濃度是決定不同水稻品種吸收鎘與糙米鎘累積濃度的主要因子，而不同期作的氣候條件與管理因素則是次要因子；從品種間的比較可確認秈稻鎘累積量遠高於稉稻，而稉稻品種間之鎘累積差異並不顯著，故相對穩定低吸收品種也皆為稉稻。期望能藉由本研究之方法篩選出穩定低鎘累積之國產水稻品種，後續再輔以適當田間農藝管理方法，將可有效降低稻米鎘累積濃度超標 (0.4 mg kg^{-1}) 之風險。

關鍵詞：鎘、水稻、GGE 雙軸圖、品種與環境交感、栽培種。

前言

人為活動引起的重金屬污染已是全球性的環境問題，對動植物的生存與人類健康造成高度風險，其中鎘為具毒性且分布廣泛的重金屬污染物，尤其環境中鎘對植物的毒性小於動物，無明顯植物屏障效應 (screen effect)，易被作物吸收後經食物鏈累積於高級消費者或人體 (Liu *et al*. 2018)。人為鎘污染來源包括：採礦、冶煉、電鍍及肥料等產業的廢水排放 (Pulford & Watson 2003)，經由灌溉系統擴散於農地土壤，大幅增加作物吸收鎘並累積於可食部位的風險 (Sebastian & Prasad 2014; Liu *et al*. 2017)。飲食習慣也是影響人類攝食

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鎘的重要因素之一，因為水稻相較其他作物對鎘的吸收累積性強，土壤鎘污染導致發生鎘米事件之議題備受關注，尤以米食為主的亞洲國家 (Tsukahara *et al*. 2003; Chen *et al*. 2018)；往昔研究顯示土壤鎘濃度偏低的農地，也可能產出鎘濃度超過 Food and Agriculture Organization/World Health Organization (FAO/WHO) 食品法典委員會 (Codex Alimentarius Commission) 標準 (> 0.4 mg kg^{-1}) 的稻米 (Chiao *et al*. 2019)，目前避免稻米生產累積超量鎘之主要策略是以降低土壤鎘的濃度及其有效性，故國內於鎘污染農地的整治工法常採行翻土稀釋法及排客土法，鎘污染濃度偏高者採現地土壤清洗法，少部分污染程度低者使用植生復育法。

然而，近年來陸續發現土壤整治後，土壤鎘濃度低於台灣環保署公告管制標準 (5.0 mg kg^{-1}) 的部分農地，仍產出鎘濃度超過台灣衛生福利部訂定的限量標準 (0.4 mg kg^{-1}) 的稻米，因應此種情況，台灣農政單位則擬定該農地以調整水稻栽培管理技術為輔導策略，常用的輔導栽培技術包括：提高土壤酸鹼度、添加土壤穩定劑及水分管理等；提高土壤酸鹼度可增加土壤對鎘的吸附能力並且降低鎘的溶解度；添加土壤穩定劑是利用其吸附、沉澱、陽離子交換或是表面交換作用以降低鎘於土壤中的移動性 (Yan *et al*. 2015; Rinklebe *et al*. 2016)；而水分管理則是透過土壤中的氧化還原電位 (Eh) 與 pH 值的改變，降低鎘在土壤中的溶解度與有效性 (Honma *et al*. 2016)。近來，除了以改善栽培管理技術達到立即且有效，以降低水稻對土壤鎘的吸收與累積，更有許多研究經由選育及推行種植低鎘吸收的水稻品種，強化水稻植株對鎘的屏障效應，以達到降低食用鎘累積過高稻米的風險 (Yu *et al*. 2006; Chiao *et al*. 2019; Chiao *et al*. 2020)；且有研究指出稉稻累積鎘的能力會低於秈稻，透過數量性狀基因座 (quantitative trait loci; QTL) 分析，發現稉稻根部液泡膜具鎘轉運蛋白 OsHMA3，能減少鎘進入木質部，增加植株對鎘的耐受性並降低鎘於穀粒的累積 (Miyadate *et al*. 2011; Ueno *et al*. 2011)。由於影響水稻累積鎘的遺傳因子眾多，評斷不同品種對鎘累積能力之差異性，仍須對相關 QTL 的遺傳多樣性提出完整的解析 (Sun *et al*. 2016)，以供未來選拔安全水稻品種的遺傳指標。

於田間收穫水稻穀粒的鎘累積濃度常顯著到受品種 (genotype; G)、環境 (environment; E) 及品種與環境 (G × E) 的交感效應所影響，所謂 G × E 的交感效應就是不同水稻品種在不同環境中的形態及生長表現差異性不一致 (Alwala *et al*. 2010)；換言之，不同品種水稻受環境影響程度不一，水稻植株吸收鎘與穀粒鎘累積濃度之品種差異性會顯著受栽培環境與管理條件的改變所影響 (Cao *et al*. 2014; Duan *et al*. 2017)。Chi *et al*. (2018) 指出，選育低鎘累積的水稻品種時應考慮 G × E 的交感效應，可經由多重環境試驗 (multiple environment trials; MET) 結果，找出高環境穩定性的低鎘累積品種。所謂 MET 是一種用於品種評估及生態群分區鑑定的試驗方法，可將試驗結果的數據以 GGE 雙軸圖分析，解析評估品種間的差異性及對不同環境效應的穩定性 (Yan *et al*. 2000; Yan 2001; Yan & Kang 2002; Dehghani *et al*. 2006)。因應目前作物品種表現的評估所需，得依賴不同地區、氣候與管理條件下試驗的資料整合 (data synthesis)，才能提出可同時評估多目標生產需求的作物表現特徵 (Brown *et al*. 2020)，以 GGE 雙軸圖的分析評估，可使 MET 資料的彙整應用不僅在說明品種、環境及兩者交感效應之影響，更可達到同時整合品種及品種與環境兩者交感效應進行品種與環境相互關聯的整合性說明，但整合說明的精確度不可避免受各資料來源之變異性與試驗設計之完整性所影響。目前，GGE 雙軸圖分析常用於評估作物產量的區域試驗結果，如：水稻 (Liu *et al*. 2016)、大麥 (Kendal 2016) 及小麥 (Kaya *et al*. 2006) 等，若作物品種在不同環境中的表現差異單純受環境效應所影響，即表示經由環境的調節控制可有效管理植株的形態與生長表現；反之，則表示品種與環境的交感效應會顯著干擾環境調節控制的主導程度。因此，若水稻植株對鎘的吸收與糙米的鎘累積濃度顯著受品種與環境的交感效應影響，欲使田間環境的調控技術 (包括：提高土壤酸鹼度、添加土壤穩定劑及水分管理) 可更有效降

低水稻植株對鎘的吸收累積，就必須釐清品種與環境的交感效應對鎘吸收累積的影響程度，並篩選出相對環境穩定性高的低鎘累積品種。為提升現有田間整治技術的效率，從降低土壤鎘污染水稻生產的目標著手，同時考量品種與栽培環境間的效應，經篩選安全水稻品種並輔以田間整治技術可降低糙米鎘濃度累積，以確保米食安全的保障。本研究以 2 處不同試驗環境與 2 個連續期作 (第一、第二期作) 對 21 種水稻栽培種進行的糙米鎘累積濃度之試驗調查資料，模擬不同水稻品種對鎘吸收累積的多環境試驗，以各品種糙米鎘累積濃度資料進行 GGE 雙軸圖分析，將品種 (G) 及品種與環境交感效應 (G × E) 對鎘累積濃度之影響總合，進行變異組成的解構分析，藉以篩選出環境穩定性高的低鎘累積品種，以供鎘污染風險潛勢較高地區的水稻安全生產品種之推薦。

材料與方法

試驗區位置及土壤鎘濃度分布

本研究試驗田區分別位於台中市后里區 (Houli; HL) (經度120°42'13"E，緯度24°18'50"N) 及彰化縣彰化市 (Changhua; CH) (經度 120°30'19"E，緯度 24°4'31"N) 的土壤高污染潛勢農地，后里試驗區農地屬紅壤的盧厝系土壤，耕作面積約為 0.25 ha；彰化試驗區農地為粘板岩及砂頁岩混合沖積土的雅溝系土壤，耕作面積約為 0.29 ha。各試驗區土壤樣本皆以土水比 1 : 1 測定 pH 值 (Mclean 1982)，利用元素分析儀測定總有機碳、比重計法測定質地 (Gee & Bauder 1986)、以 1 M 醋酸銨法測定陽離子交換容量 (Rhoades 1982)，並利用 0.1 M 鹽酸 (Nelson *et al*. 1959) 及微波輔助王水消化法萃取有效性鎘 (Cd_a) 及總鎘 (Cd_t)，基本性質列於表 1。2 試驗區 HL 與 CH 的表層土壤 (0–15 cm) Cd_a 平均分別為 2.73 及 0.13 mg kg^{-1}，Cd_t 平均分別為 3.18 及 0.25 mg kg^{-1}，而且兩試驗區內 Cd_t 的空間分布變異如圖 1、圖 2 所示。

供試品種及田間試驗設計

本研究以 21 種台灣地區主要栽培種水稻為供試品種，包括：「台稉 2 號」(‘TK2’)、「台稉 8 號」(‘TK8’)、「台稉 9 號」(‘TK9’)、「台稉 14 號」(‘TK14’)、「台稉 16 號」(‘TK16’)、「桃園 3 號」(‘TY3’)、「台農 71 號」(‘TNG71’)、「台中 192 號」(‘TC192’)、「台南 11 號」(‘TN11’)、「高雄 139 號」(‘KH139’)、「高雄 145 號」(‘KH145’)、「高雄 147 號」(‘KH147’)、「台東 30 號」(‘TT30’)、「台東 33 號」(‘TT33’)、「台中秈 10 號」(‘TCS10’)、「台中秈 17 號」(‘TCS17’)、「高雄秈 7 號」(‘KHS7’)、「台農糯 73 號」(‘TNGW73’)、「台稉糯 1 號」(‘TKW1’)、「台稉糯 3 號」(‘TKW3’) 以及「台中秈糯 2 號」(‘TCSW2’)。在兩試驗田依據土壤鎘濃度分布梯度變化方向進行插秧，植株行排列方向與濃度梯度方向垂直 (圖 1 及圖 2)，每行種植單一品種，將可供說明土壤鎘濃度背景差異對各品種水稻吸收鎘的影響。然而，因本文所採納之試驗設計在品種間僅排列 1 次，無品種間重複隨機排列，為單次重複處理組合，各品種內之土壤變異若大於品種間遺傳變異，可能致使品種間多重檢定效力大幅降低 (Filazzola & Cahill 2021)。

為說明水稻生長及稻穀鎘累積受季節性氣候差異 (氣溫及水情) 之影響，本研究於 2 試驗區各進行連續 2 期作的試驗調查，以瞭解期作間的差異，試驗區 HL 的期程包括 2016 年

表 1. 試驗地區土壤基本性質。

Table 1. Physical and chemical properties of the studied soils.

Site	pH	SOM[z] (%)	CEC[y] [cmole (+) kg^{-1}]	Cd_t[x] (mg kg^{-1})	Cd_a[w] (mg kg^{-1})	Texture
Houli (HL)	6.26	2.19	5.39	3.18	2.73	Loamy
Changhua (CH)	6.25	1.64	6.94	0.25	0.13	Loamy

[z] SOM: soil organic matter.
[y] CEC: cation exchange capacity.
[x] total Cd concentration
[w] available Cd concentration

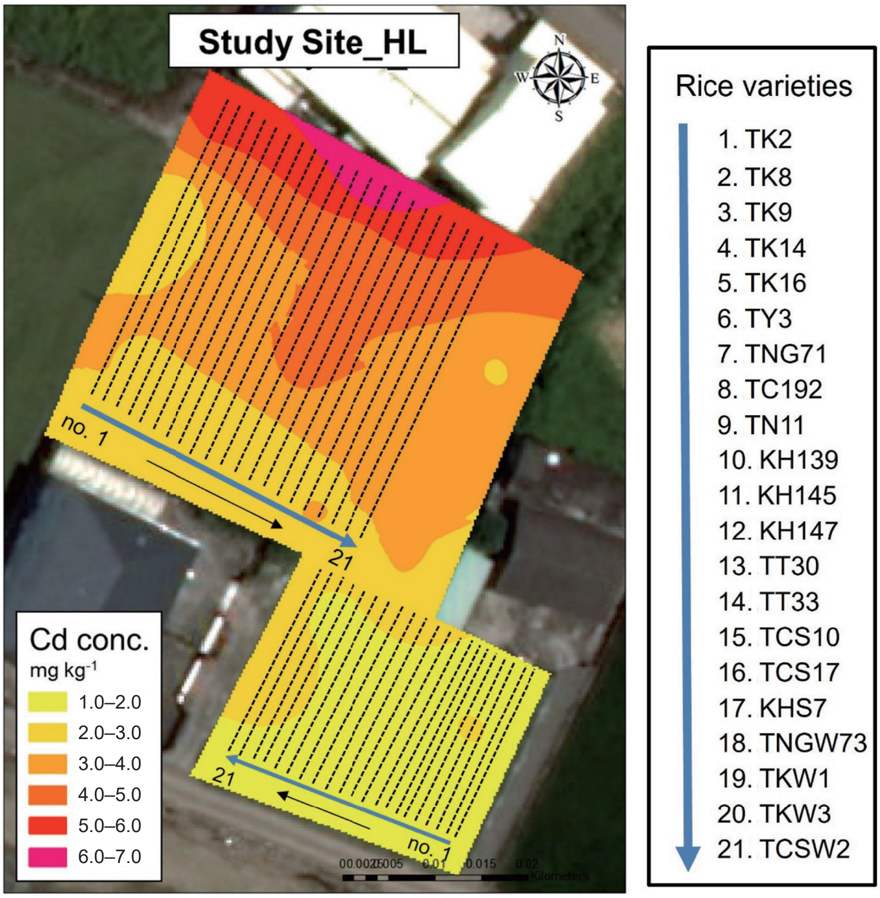


圖 1.　后里試驗區 (HL) 土壤表土鎘總量濃度 (mg kg^{-1}) 分布圖。

Fig. 1.　Spatial distribution of soil total Cd concentrations (mg kg^{-1}) in the Houli (HL) study site.

第一期作 3 月初至 6 月底，第二期作 7 月底至 11 月中；試驗區 CH 的期程包括 2017 年第一期作 2 月底至 6 月底，第二期作 7 月底至 11 月中。各期作之氣象資料取自中央氣象局網站觀測資料查詢系統 (https://e-service.cwb.gov.tw/HistoryDataQuery/index.jsp)，試驗區 HL 的月均溫及雨量紀錄依據台中市后里測站資料 (經度 120°43'47''E，緯度 24°18'37''N)，月累積日照時數紀錄依據苗栗區農業改良場測站資料 (經度 120°49'45''E，緯度 24°29'45''N)；試驗區 CH 的月均溫及雨量紀錄依據彰化縣鹿港測站資料 (經度 120°25'49''E，緯度 24°4'31''N)，月累積日照時數紀錄依據彰化縣台中區農業改良場測站資料 (經度 120°32'4''E，緯度 24°0'11''N)。

根圈土壤與植體的樣本製備分析

於水稻成熟期採集水稻植株及其根圈土壤樣本，每個品種採樣數皆為 6 重複，因此各試驗區單一期作共採集 126 組 (21 × 6) 根圈土壤與植株樣本。採集的稻穀置入烘箱以 70℃ 烘乾 48 h 後，先脫殼碾製成糙米，再以鈦刀磨粉機粉碎，置入乾淨夾鏈袋備用；根圈土壤樣本先經風乾並以木桿製磨粉碎後，過 0.5 mm 標準篩網裝罐備用，以供分析土壤基本性質及總鎘濃度。粉碎糙米樣本以硝酸-雙氧水 (10：1，v/v) 進行微波消化，經感應耦合電漿質譜儀 (ICP-MS, 7500C, Agilent, Santa Clara, CA, USA) 檢測消化液之鎘濃度。0.1 M 鹽酸之萃取溶液及王水消化溶液皆以感應耦合電

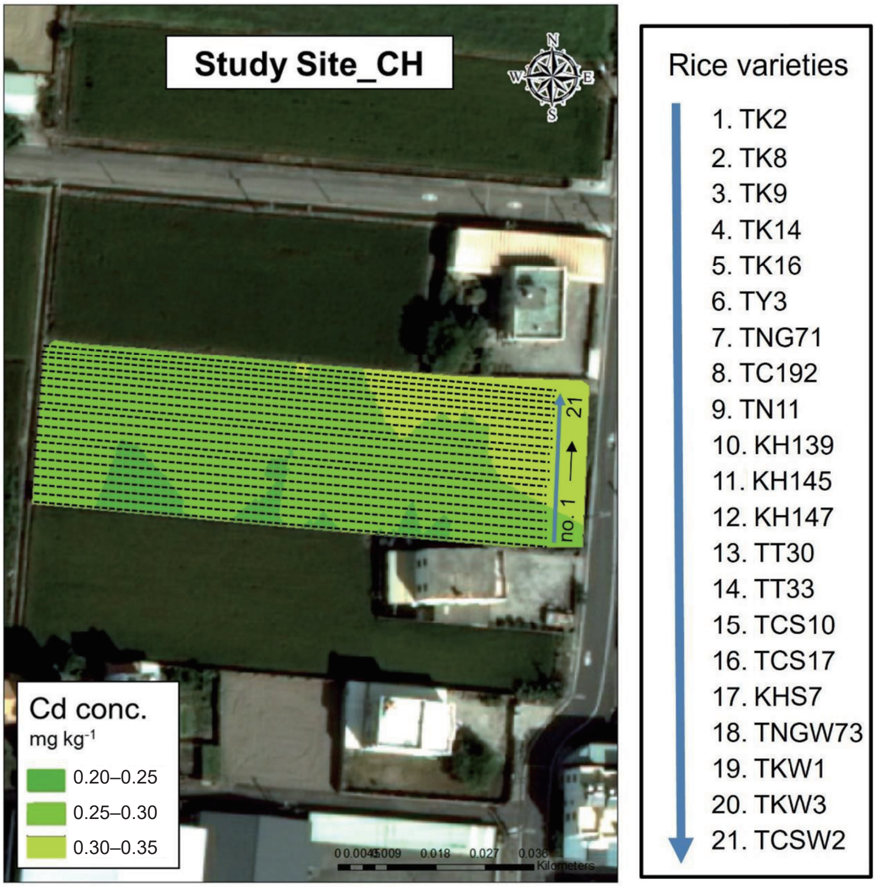


圖 2. 彰化試驗區 (CH) 土壤表土鎘總量濃度 (mg kg^{-1}) 分布圖。

Fig. 2. Spatial distribution of soil total Cd concentrations (mg kg^{-1}) in the Changhua (CH) study site.

漿光譜儀 (ICP-OES, Ultima 2C, Horiba Jobin Yvon, Longjumeau, France) 測定土壤樣本 Cd_a 與 Cd_t。2 處試驗區各 2 期作共取得 4 組糙米與根圈土壤鎘濃度資料，再經由糙米 (C_{grain}) 及根圈土壤鎘濃度 (C_{soil})，可間接計算生物濃縮因子 (bioconcentration factor; *BCF*)，如式 (1) 所述，並以變方分析 (analysis of variance; ANOVA) 分別檢定各組 C_{grain} 與 *BCF* 資料在品種間變異之顯著性，再以最小顯著差異法 (least significant difference; LSD) 進行品種間多重比較的檢定。

$$BCF = \frac{C_{grain}}{C_{soil}} \text{。} \quad (1)$$

GGE 雙軸圖分析

依據本研究的試驗設計，糙米鎘濃度的變異分析可表示成下述的線性模式：

$$Y_{ijk} = \mu + g_i + e_j + ge_{ij} + \varepsilon_{ijk} \quad (2)$$

其中 μ 為樣品平均值，g_i 代表第 i (i = 1,..., g) 品種的主效應 (G)，e_j 代表第 j (j = 1,..., e) 環境之主效應 (E)，ge_{ij} 表示第 i 品種與第 j 環境的交感效應 (G × E)，ε_{ijk} 為誤差項。試驗資料先進行變方分析，判定 G × E 是否達顯著，若達顯著時，可對 G + G × E 進行 GGE 雙軸圖分析探討 (Yan *et al*. 2000)。接著，將式 (2) 改寫為式 (3) ，再進行奇異值 (singular value) 解析：

$$Y_{ijk} - \mu - e_j = (g_i + ge_{ij}) + \varepsilon_{ijk} = \sum_{n=1}^{N} \xi_{in}(\lambda_n \eta_{jn}) + \varepsilon_{ijk} \quad (3)$$

其中 Y_{ijk} 表示品種 i 在環境 j 及重複 k 中的數值，μ 為樣品總均值，λ_n 為第 n 個 [n = 1, ... , N and N = min (g, e)] 奇異值，ξ_{in} 為品種 i 對應第 n 個奇異向量 (singular vector) 之數值，η_{jn} 為環境 j 在第 n 個奇異向量之數值 (Liu *et al*. 2016)；上述之奇異值解析與 GGE 分析流程皆以軟體 R 撰寫演算程式進行。GGE 雙軸圖分析是利用上述奇異值解析後產生最具代表品種主效應 (G) 及品種與環境的交感效應 (G × E) 總和變異之 2 個垂直的主成分向量軸 (第一與第二奇異向量)，再以代表各品種與試驗環境的平均數值投影至 2 個主成分向量軸的分數 (scores) 繪製散布圖 (即是 GGE 雙軸圖)。GGE 雙軸圖分析的操作程序是先從雙軸圖原點對不同環境得分散布點，分別畫出代表試驗環境的向量及代表向量平均的平均環境軸，再計算繪出不同品種的分數散布點位置與平均環境軸的垂直距離，藉垂直距離長短說明各品種受環境影響程度之差異，距離短代表品種性狀表現的環境穩定性高，垂直距離愈長代表環境穩定性較低，而且不同試驗環境的向量箭頭所指向之特定品種得分位置，即代表該品種在此試驗環境具有累積較高糙米鎘濃度的性狀表現。

結果與討論

不同試驗環境對水稻糙米鎘累積濃度之影響

經本研究彙整分析 2 處試驗區之一、二期作水稻試驗期間氣象資料得知，后里試驗區 (HL) 第一期作平均氣溫為 23.28℃，累積雨量為 897 mm，累積日照時數為 596.7 h，第二期作平均氣溫為 25.08℃，累積雨量為 484.5 mm，累積日照時數為 651.1 h；彰化試驗區 (CH) 第一期作平均溫度為 24.03℃，累積雨量為 720 mm，累積日照時數為 579.2 h，第二期作平均氣溫為 26.73℃，累積雨量為 158.5 mm，累積日照時數為 604.3 h。上述資訊顯示，相較於 CH，HL 均溫較低，但累積雨量與累積日照時數皆較高；而相較第一期作，第二期作的均溫較高且累積日照較長，但累積雨量較低。再者，表 1 所述之兩處試驗區土壤有機質 (soil organic matter; SOM) 與陽離子交換 (cation exchange capacity; CEC) 差異顯著，而且兩處土壤鎘之有效性 (Cd_a) 與總量 (Cd_t) 差異極大。因此，本研究欲整合不同期作氣候條件與土壤鎘濃度背景顯著差異之試驗區資料，模擬於栽培生產條件顯著差異之不同試驗環境取得之水稻糙米累積鎘之 MET 資料，藉以討論水稻水稻吸收累積鎘的品種差異性與環境交感效應。依上述期作別與試驗區之氣候與土壤鎘濃度的變異特性，分別將后里一、二期作及彰化一、二期作設定為 4 種試驗環境 (H1、H2、C1 及 C2)，並假設 4 者間的試驗條件足以表示水稻栽培生產之環境差異，可藉以討論糙米鎘累積性的品種差異與環境交感效應，並進行 GGE 雙軸圖分析。

圖 3 所示為后里 (HL) 與彰化 (CH) 試驗區 2 期作各品種水稻糙米及根圈土壤之鎘濃度分布，於 HL 第一期作，各品種間根圈土壤鎘濃度無顯著差異，平均約 2.80 mg kg^{-1}，其糙米鎘濃度分布範圍 1.58–4.23 mg kg^{-1}，平均值 2.66 mg kg^{-1}，所有供試品種的糙米鎘濃度皆超過衛生福利部制定的限量標準 0.4 mg kg^{-1}，尤以 ‘TCS10’、‘TCSW2’ 及 ‘TK2’ 糙米最高 (3.88–4.23 mg kg^{-1})，‘TKW1’、‘KH139’ 及 ‘TT30’ 糙米相對較低 (1.58–1.74 mg kg^{-1})。在 HL 第二期作，各品種間根圈土壤鎘濃度亦無顯著差異，平均值 3.02 mg kg^{-1}；糙米鎘濃度則介於 1.55–6.84 mg kg^{-1}，平均較第一期作高，以 ‘TCS10’、‘TCS17’ 及 ‘TCW2’ 糙米最高 (6.68–6.84 mg kg^{-1})， 而 ‘TKW3’、‘TT30’ 及 ‘TK2’ 糙米相對較低 (1.55–1.69 mg kg^{-1})。再者，於 CH 第一期作各品種糙米鎘濃度介於 0.22–0.67 mg kg^{-1}，平均值為 0.38 mg kg^{-1}，但有 9 個品種糙米鎘濃度超過限量標準，其中 ‘TCS17’、‘TK14’ 及 ‘TCSW2’ 糙米鎘濃度最高，介於 0.45–0.67 mg kg^{-1}，而 ‘TKW1’、‘TT33’ 及 ‘KH139’ 糙米鎘濃度最低 (0.22–0.30 mg kg^{-1})；在 CH 第二期作，僅 4 個品種超過限量標準，‘TK9’ 的糙米鎘濃度最高 (0.69

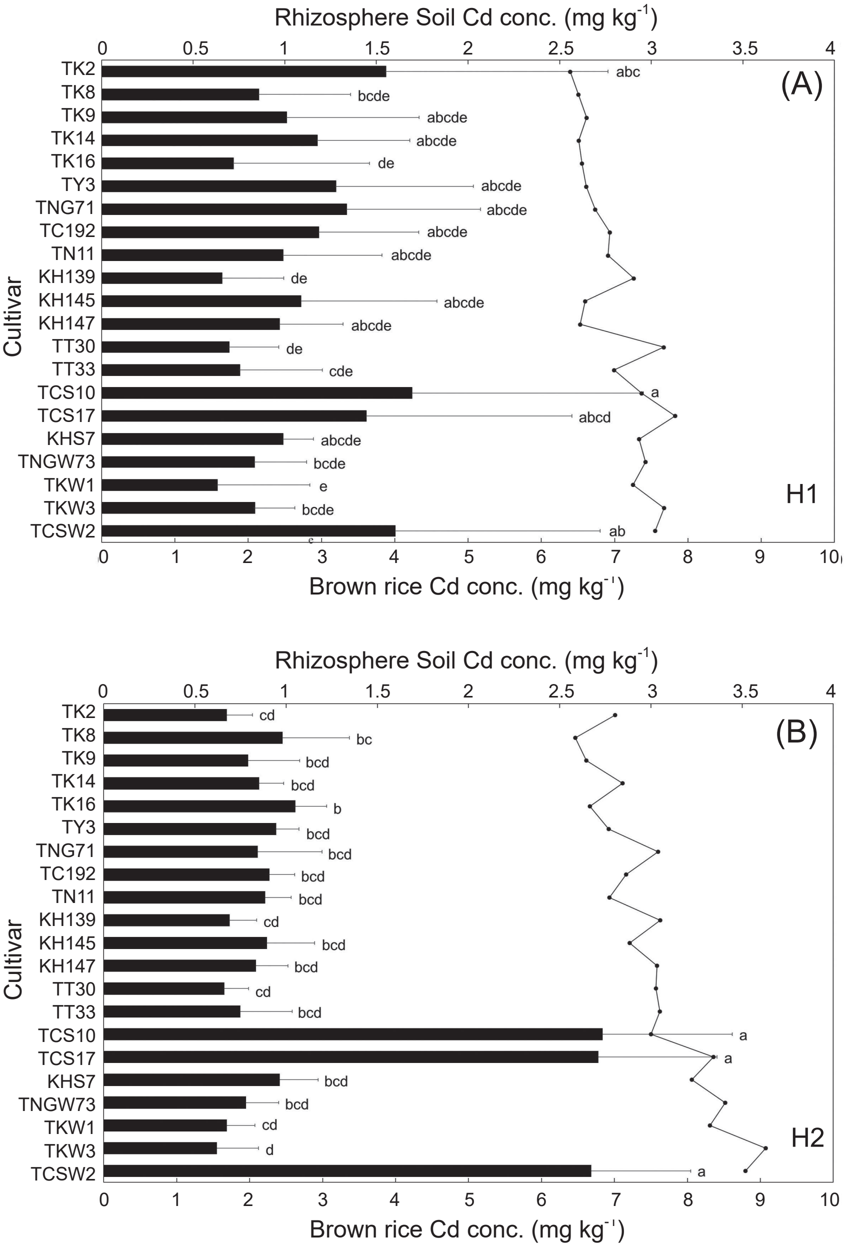
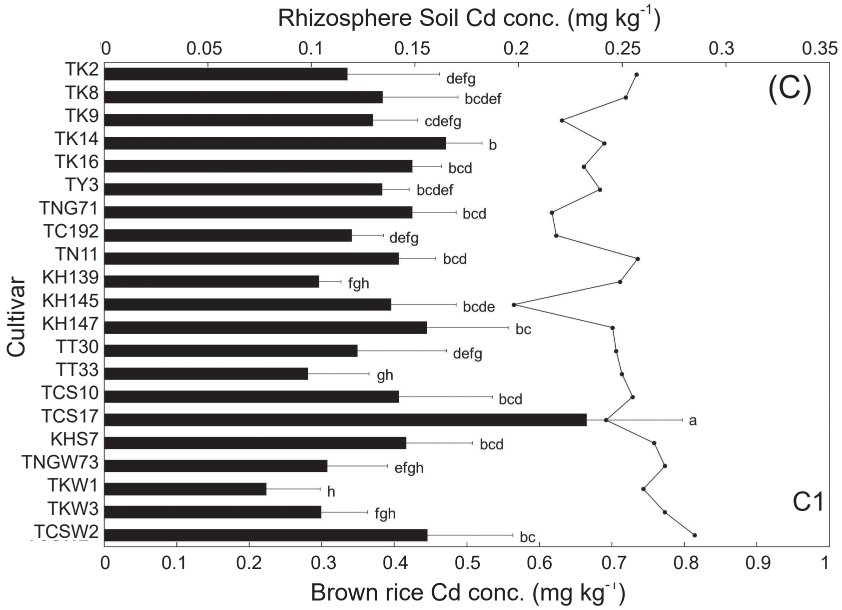


圖 3. 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米及其根圈土壤鎘濃度 (mg kg^{-1}) 的差異分別如長條與折線圖所表示：后里 (HL) 試驗區 (A) 第一期作及 (B) 第二期作與彰化 (CH) 試驗區 (C) 第一期作及 (D) 第二期作。柱狀圖誤差棒表示各品種平均值的標準差 ($n = 6$)，有英文字母相同者表示彼此間平均值差異以最小顯著差異法 (LSD) 檢定不顯著 ($P < 0.05$)。

Fig. 3. Profiles of Cd concentrations (mg kg^{-1}) of brown rice and rhizosphere soil drawn with bar and line charts, respectively, for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’): the (A) first and (B) second crops in the Houli (HL) site and the (C) first and (D) second crops in the Changhua (CH) site. Error bars represent the standard deviations of the mean values ($n = 6$). Same letters above the bars indicate the mean values are not significantly different by least significant difference (LSD) test ($P < 0.05$).



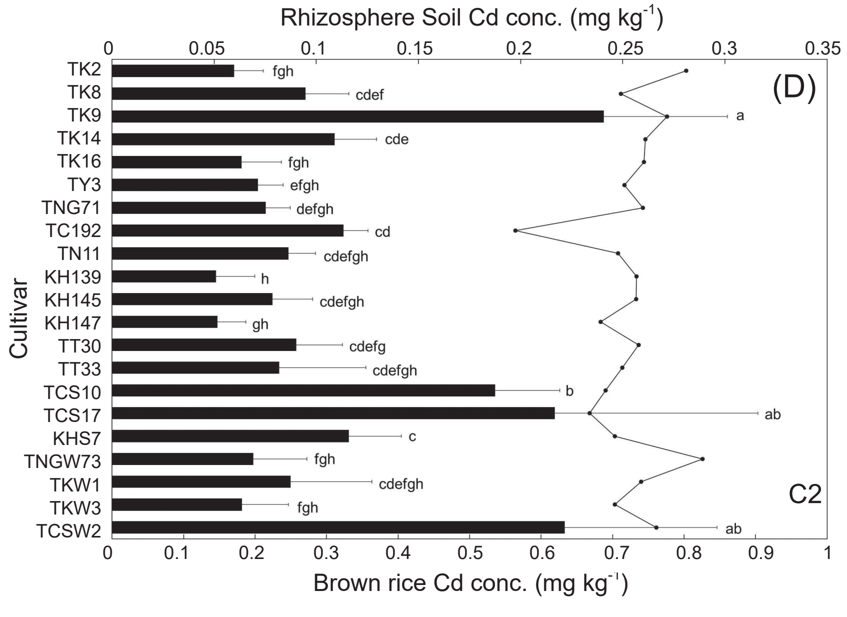


圖 3. 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米及其根圈土壤鎘濃度 (mg kg^{-1}) 的差異分別如長條與折線圖所表示：后里 (HL) 試驗區 (A) 第一期作及 (B) 第二期作與彰化 (CH) 試驗區 (C) 第一期作及 (D) 第二期作。柱狀圖誤差棒表示各品種平均值的標準差 ($n = 6$)，有英文字母相同者表示彼此間平均值差異以最小顯著差異法 (LSD) 檢定不顯著 ($P < 0.05$)。(續)

Fig. 3. Profiles of Cd concentrations (mg kg^{-1}) of brown rice and rhizosphere soil drawn with bar and line charts, respectively, for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’): the (A) first and (B) second crops in the Houli (HL) site and the (C) first and (D) second crops in the Changhua (CH) site. Error bars represent the standard deviations of the mean values ($n = 6$). Same letters above the bars indicate the mean values are not significantly different by least significant difference (LSD) test ($P < 0.05$). (continued)

mg kg^{-1})，其次 ‘TCSW2’、‘TCS17’ 及 ‘TCS10’，糙米鎘濃度介於 0.54–0.63 mg kg^{-1}，其餘品種無顯著差異且糙米鎘濃度介於 0.15–0.33 mg kg^{-1}。

以上結果顯示，試驗區 HL 與 CH 於第二期作的糙米鎘濃度平均皆高於第一期作，推測可能第二期作的均溫較高且累積日照較長，但累積雨量卻較低，在固定淹灌頻率的管理條件下可能使田間現地處於間歇湛水的土壤境況，土壤若局部呈現氧化狀態將促使鎘物種轉變成移動性高的硫酸鎘，進而提高稻穀的鎘累積量 (Arao *et al*. 2009; Syu *et al*. 2019)。比較兩試驗區糙米鎘累積結果發現，糙米鎘濃度與根圈土壤鎘濃度在試驗區 HL 皆遠高於試驗區 CH，兩試驗區間糙米與根圈土壤鎘濃度差異皆近 10 倍之多 (圖 3)，該結果顯然與兩區土壤的鎘濃度差異有關 (表 1)；而且試驗區 HL 灌溉水源不足，灌溉頻率較低 (5.5 d 輪灌 1 次)，導致間歇湛水日數縮短，致使田區土壤鎘有效性升高。Duan *et al*. (2017) 指出抽穗期較早的水稻品種於充實期維持湛水狀態的水分管理，稻穀的鎘累積濃度會比抽穗期較晚的品種顯著低。此外，品種間糙米鎘濃度之結果顯示，秈稻對於鎘累積能力普遍高於稉稻，這與 Liu *et al*. (2005) 的研究結果相符，但部分品種 (如 ‘TK9’、‘TK16’) 在不同環境中稻穀鎘累積濃度則變異較大，Chi *et al*. (2018) 的研究也提出論述，稉型稻對土壤鎘的累積能力確實低於秈型稻與雜交稻。因此，本研究於圖 3 所示之 4 組試驗環境確實對各品種糙米鎘濃度之差異性造成影響，彙整 4 組資料的結果可以歸納發現 HL 試驗區的糙米鎘濃度顯著高於 CH 試驗區，而 HL 試驗區一期作各品種糙米鎘濃度間的變異性較二期作大，但 CH 試驗區二期作各品種糙米鎘濃度間的變異性較一期作大，表示各品種糙米鎘濃度在 4 組環境試驗的差異性大，也就是不同試驗環境條件對各品種的影響不一致，確實有必要進一步以 GGE 分析將環境條件的影響標準化，再討論糙米鎘累積的品種差異性。

再者，依據 BCF 的定義：植物器官與土壤重金屬濃度的比值，其可用於評估植物自土壤中累積重金屬的能力 (Song *et al*. 2015; Xie *et al*. 2015)；本研究以糙米與根圈土壤的鎘濃度比值計算 BCF，結果如圖 4，於兩處試驗區皆呈現秈型稻品種的 BCF 值多高於稉型稻品種，此結果與 Li *et al*. (2019) 的研究結果相近。然而，在環境條件差異及品種與環境交感效應影響之下，各水稻品種在不同環境的 BCF 值大小順序並無一致性，試驗區 HL 第一期作的 ‘TK2’ 鎘累積能力最高，而試驗區 CH 第二期作的稉型稻以 ‘TK9’ 與 ‘TC192’ 鎘累積能力最高，則與秈型稻無顯著差異，但 ‘KH139 與 ‘TKW1’ 在不同試驗區與期作皆顯示較低的鎘累積能力。以上結果顯示，在不同環境中以 BCF 值能觀察說明鎘累積能力的品種變異性，可用於比較秈型及稉型稻間之差異，並簡單分類出相對低鎘累積品種，如：‘TKW1’、‘KH139’、‘TT30’、‘TKW3’ 及 ‘TT33’，以及高鎘累積品種 ‘TCS10’、‘TCS17’ 及 ‘TCSW2’；尤其 BCF 可凸顯不同期作因氣候條件不同影響各品種水稻鎘累積之差異性，但可能就不易說明各水稻品種在不同污染程度土壤環境的鎘累積差異。基於田間管理的實際情境，不同期作的氣候條件與不同污染程度的土壤環境差異對各品種糙米累積鎘的影響並非獨立互不干擾，導致即使於相同期作，各品種因為栽種田區的土壤污染程度與氣候條件不同，其糙米鎘累積濃度可能差異甚大，故 BCF 仍無法明確歸納這些品種在不同環境中是否具有穩定的低或高鎘累積能力。

品種與環境交感效應對糙米鎘濃度之影響評估

圖 5 所示為 GGE 雙軸圖分析結果，利用 GGE 分析法對不同水稻品種在兩處試驗區一、二期作吸收土壤鎘的累積情形進行討論，依式 (3)，將各品種之糙米鎘濃度資料先扣除環境主效應，保留品種效應及品種與環境之交感效應，再經奇異值解析求出 2 個主成分 PC1 及 PC2，其分別可解釋 71.86% 及 12.18% 的變異組成，即是以 PC1 與 PC2 可以說明本試驗資料的變異達 84.04%。圖 5 中以點虛線表示的環境向量愈長，代表該試驗環境的各品種糙米鎘濃度間之差異性愈大，亦即在環境向量愈長的試驗條件下愈能分辨出品種間之差異性，兩處試驗區二期作的 H2 與 C2 環境向量較長，

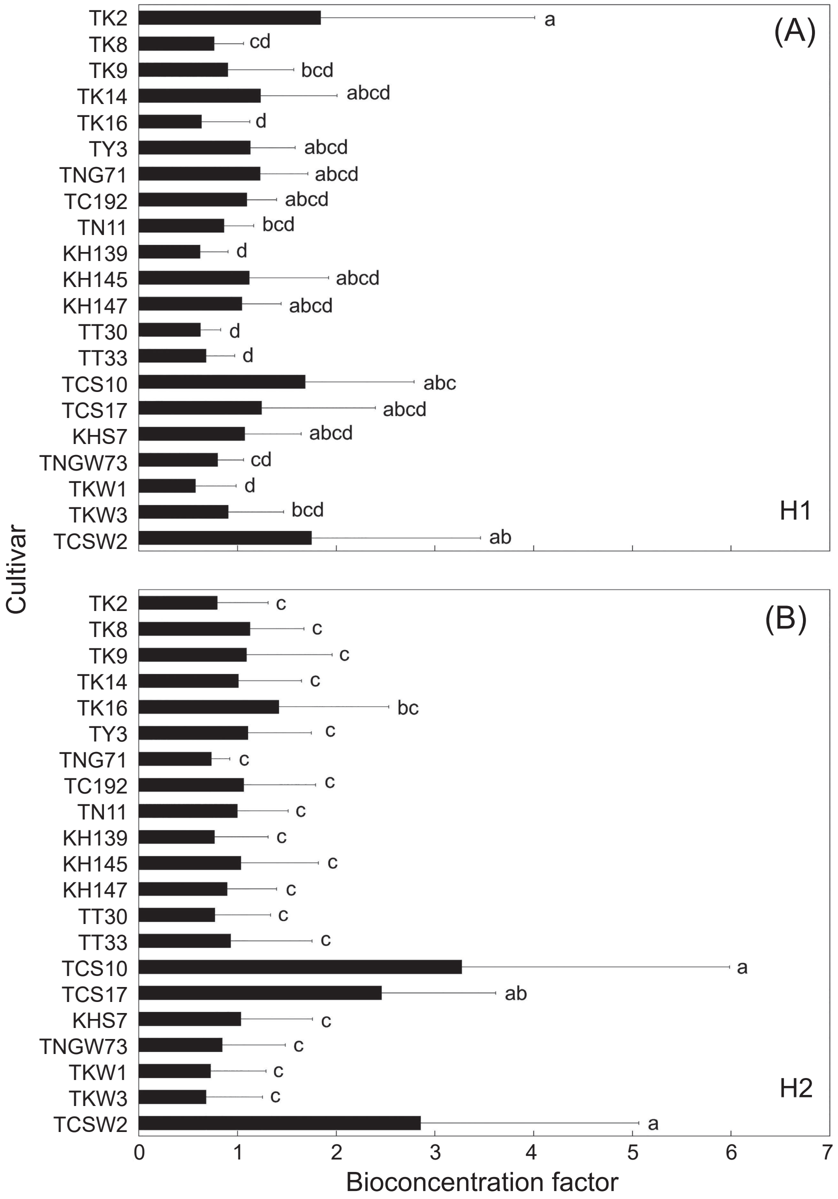


圖 4. 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米的鎘生物濃縮因子 (BCF)：后里 (HL) 試驗區 (A) 第一期作及 (B) 第二期作與彰化 (CH) 試驗區 (C) 第一期作及 (D) 第二期作。柱狀圖誤差棒表示各品種平均值的標準差 (n = 6)，有英文字母相同者表示彼此間平均值差異以最小顯著差異法 (LSD) 檢定不顯著 (P < 0.05)。

Fig. 4. Profiles of Cd bioconcentration factor (BCF) by brown rice for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’): the (A) first and (B) second crops in the Houli (HL) site and the (C) first and (D) second crops in the Changhua (CH) site. Same letters above the bars indicate the mean values are not significantly different by least significant difference (LSD) test (P < 0.05).

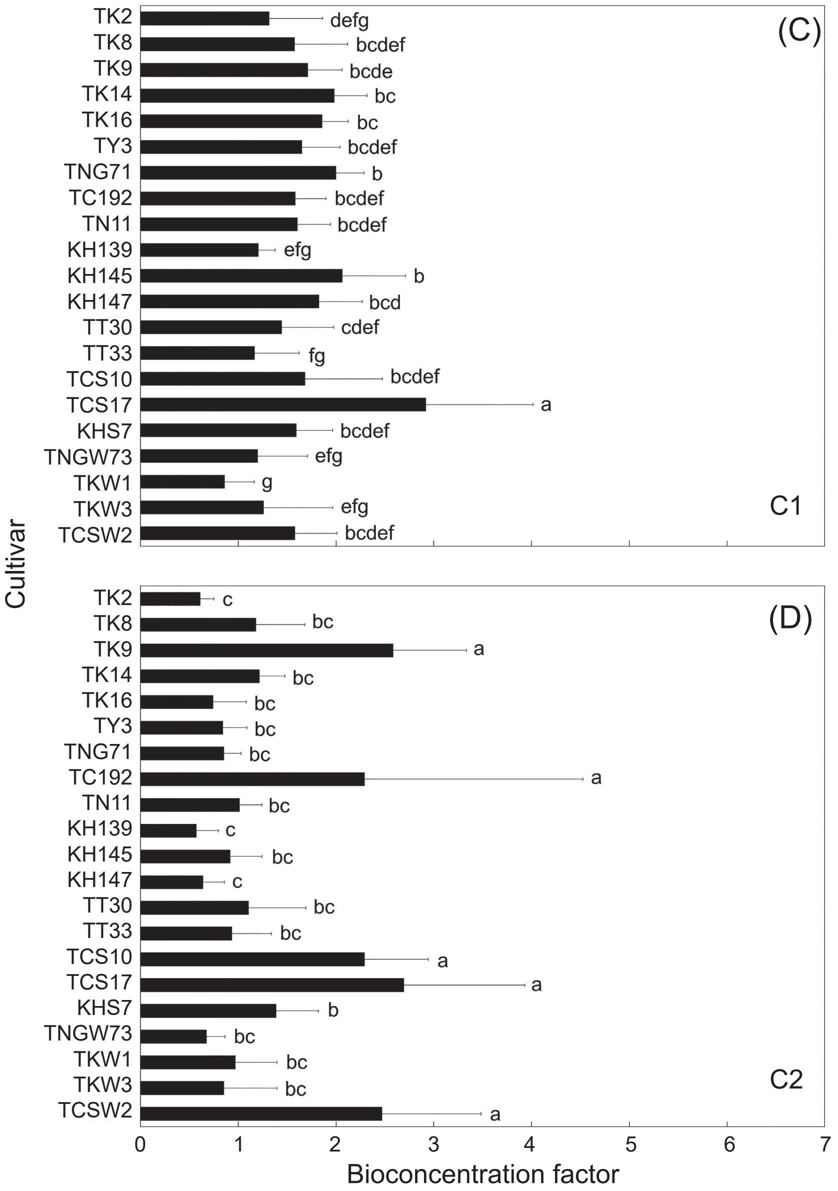


圖 4.　21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米的鎘生物濃縮因子 (BCF)：后里 (HL) 試驗區 (A) 第一期作及 (B) 第二期作與彰化 (CH) 試驗區 (C) 第一期作及 (D) 第二期作。柱狀圖誤差棒表示各品種平均值的標準差 (n = 6)，有英文字母相同者表示彼此間平均值差異以最小顯著差異法 (LSD) 檢定不顯著 (P < 0.05)。(續)

Fig. 4.　Profiles of Cd bioconcentration factor (BCF) by brown rice for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’): the (A) first and (B) second crops in the Houli (HL) site and the (C) first and (D) second crops in the Changhua (CH) site. Same letters above the bars indicate the mean values are not significantly different by least significant difference (LSD) test (P < 0.05). (continued)

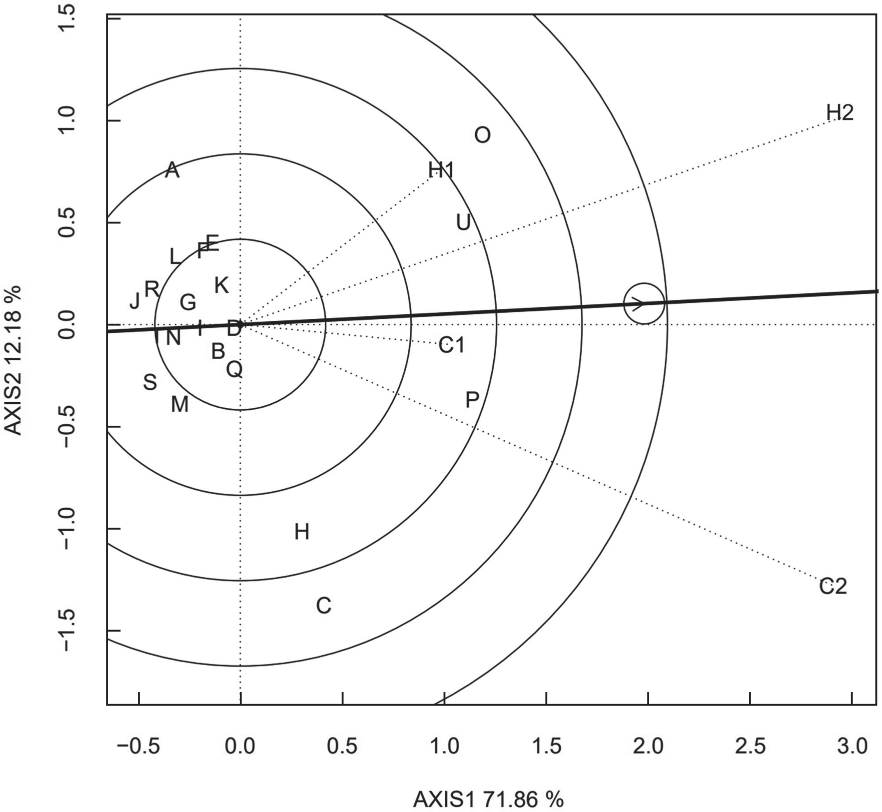


圖 5. 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米鎘濃度之 GGE 雙軸圖；各水稻品種代號依序如下列：A、B、C、D、E、F、G、H、I、J、K、L、M、N、O、P、Q、R、S、T 及 U。后里 (HL) 試驗區第一與第二期作的環境向量終點分別標示為 H1 與 H2；彰化 (CH) 試驗區第一與第二期作的環境向量終點分別標示為 C1 與 C2；小圓圈代表環境向量和的平均環境座標，粗實線為通過座標軸原點以及平均環境座標的平均環境軸，箭頭方向代表糙米鎘累積量較高的環境條件。

Fig. 5. GGE bi-plot of brown rice Cd concentrations for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’), which are denoted as the capital letters, A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, and U, respectively. The environment vectors drawn with dot lines for the first and second crops in the Houli (HL) site are denoted as H1 and H2; the environment vectors drawn with dot lines for the first and second crops in the Changhua (CH) site are denoted as C1 and C2. The small open circle represents the sum of environment vectors as the average of environments, the bold solid line is the average axis of environments, and the arrow directs the environmental tendency toward Cd accumulation in brown rice.

故於二期作進行試驗較可顯著鑑別各品種糙米鎘累積濃度之差異，將有利於汰除表現不穩定之品種；然而期作效應係屬每年每期作逢機遭遇氣候變化之情境，第一與第二期作的溫度、日長變化趨勢明顯迥異，本研究團隊將持續收集更多年度相同期作的試驗資料，藉以釐清跨年度同一氣候情境的品種間變異幅度。此外，Phuke *et al*. (2017) 以 GGE 分析進行篩選試驗建議，試驗環境向量與平均環境軸的夾角愈小即表示該試驗環境具備多環境試驗條件的代表性，如圖 5 的 C1 向量與平均環境軸夾角最小，最具代表多環境試驗條件的環境特性，相較 C2、H1 及 H2，更適合於評估比較表現穩定的品種。

圖 6 為各水稻品種糙米鎘濃度的生態群分區 (mega-environments; MEs) 評估，是由距離

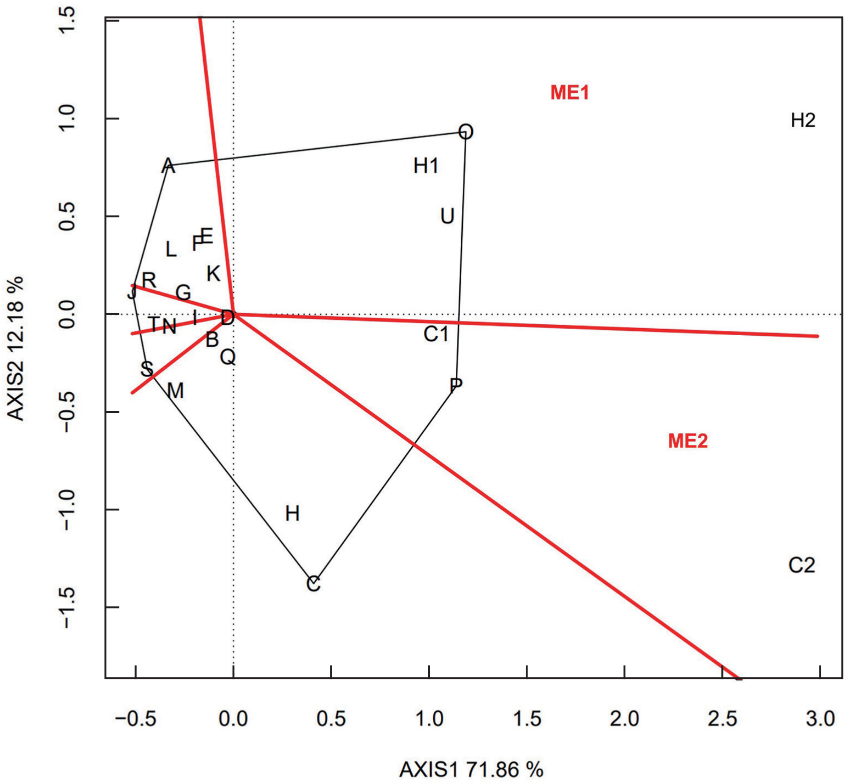


圖 6. 以 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米鎘濃度的 GGE 雙軸圖進行生態群分區評估。各水稻品種代號依序如下列：A、B、C、D、E、F、G、H、I、J、K、L、M、N、O、P、Q、R、S、T 及 U；后里 (HL) 試驗區第一與第二期作分別標示為 H1 與 H2；彰化 (CH) 試驗區第一與第二期作分別標示為 C1 與 C2。由距離座標軸原點最遠的品種向量相連成多邊形，實線為垂直於多邊形各邊並通過座標軸原點的垂直線，可將 H1、H2、C1 與 C2 試驗環境界定於 2 個虛擬生態環境 (ME1 與 ME2)。

Fig. 6. Assessment of ecological regionalization with the GGE bi-plot of brown rice Cd concentrations for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’), which are denoted as the capital letters, A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, and U, respectively. The first and second crops in site Houli (HL) are denoted as H1 and H2; the first and second crops in site Changhua (CH) are denoted as C1 and C2. The connected polygon of the cultivars farthest from the origin. The red lines are perpendicular to the edges of the polygon to divide the tested environments (i.e., H1, H2, C1, and C2) into roughly two mega-environments (M1 and M2).

原點最遠的品種 (A、J、S、C、P 及 O) 連接成為包圍所有品種代號之多邊形，並繪製垂直多邊形各邊且通過原點的紅色直線，這些直線將繪圖區域劃分為若干區塊，若區塊包含 1 個多邊形的角，則代表位於此角的品種投影至該區塊內之各環境向量均有最長的投影，表示此品種在該區塊的各環境中皆有最高表現，換言之就是此品種糙米鎘濃度最高。Yan *et al.* (2000) 將這些有相同最高表現品種的環境區塊稱為生態群，在這些環境可以採用類似的品種推廣策略，此法已被 Liu *et al.* (2016) 用於作物產量的區域試驗，並成功篩選出在不同環境中的高產品種。根據圖 6 所示，垂直於 ‘TK2’–‘TCS10’ (A–O) 及 ‘TCS10’–‘TCS17’ (O–P) 的 2 條紅線範圍間涵蓋環境向量 H1 與 H2，且涵蓋品種 O (‘TCS10’) 與 U (‘TCSW2’)，表示 H1

與 H2 屬於相同生態群分區 ME1，糙米鎘累積量最高品種 ‘TCS10’ 與 ‘TCSW2’；表示此 2 個品種在后里環境中有較高的糙米鎘累積風險、垂直於 ‘TCS10’–‘TCS17’ (O–P) 及 ‘TK9’–‘TCS17’ (C–P) 的 2 條紅線範圍間涵蓋環境向量 C1 與 C2，且涵蓋品種 P (‘TCS17’)，表示 C1 與 C2 在相同生態群分區 ME2，糙米鎘累積量最高品種 ‘TCS17’。然而，本研究目前因為試驗環境較少，生態群分區 ME1 與 ME2 僅分別區隔出兩處試驗環境 HL 與 CH 的土壤條件效應，卻未顯示出兩期作間氣候條件效應的區隔，未來若能加入更多其他試驗環境在不同期作的資料，將更有利於篩選辨識出受期作氣候條件影響的高污染風險品種。

水稻穀粒累積鎘的品種變異與環境穩定性評估

為評估說明各品種糙米鎘濃度在不同試驗環境的差異性與穩定性，以圖 5 中各試驗環境向量 (H1、H2、C1 及 C2) 為投影軸，將各品種向量投影至軸上，分別如圖 7A、7B、7C 及 7D 所示之結果，投影點愈靠右者表示此品種在該試驗區的糙米鎘濃度愈高，且圖中虛線標示各品種對應於環境向量軸的投影距離，距離愈遠者表示在該試驗區的糙米鎘濃度變異性愈大，故各品種投影至環境向量軸的距離可視為品種表現的環境穩定性指標，距離愈近則穩定性愈高，反之則否。圖 7A 與 7B 顯示，在后里試驗區 ‘TCS10’ (編號 O)、‘TCSW2’ (編號 U) 及 ‘TCS17’ (編號 P) 之糙米鎘濃度最高，而且 ‘TCSW2’ 在一、二期作 (H1 與 H2) 的穩定性皆高於 ‘TCS10’ 與 ‘TCS17’，是后里區環境穩定性較高的高鎘累積品種；相對而言，‘TKW1’ (編號 S)、‘TT30’ (編號 M)、‘KH139’ (編號 J)、‘TKW3’ (編號 T) 等品種糙米鎘濃度較低，而且兩期作的穩定性皆相對較低，是后里地區環境穩定性較高的低鎘累積品種。圖 7C 與 7D 顯示，在彰化試驗區 ‘TCS17’ (編號 P) 之糙米鎘濃度最高，其次 ‘TCS10’ (編號 O)、‘TK9’ (編號 C)、‘TC192’ (編號 H) 及 ‘TCSW2’ (編號 U)，而且 ‘TCS17’ 在一、二期作 (C1 與 C2) 的穩定性也最高，是彰化區環境穩定性較高的高鎘累積品種；而 ‘TK2’ (編號 A)、‘KH139’ (編號 J)、‘KH147’ (編號 L)、‘TNGW73’ (編號 R)、‘TKW1’ (編號 S)、‘TKW3’ (編號 T) 等品種糙米鎘濃度較低，且 ‘KH139’、‘KH147’、‘TNGW73’ 及 ‘TKW3’ 在兩期作的穩定性相對較高，算是彰化區環境穩定性較高的低鎘累積品種。

圖 8 所示為各品種投影至平均環境軸上的投影距離與投影點分布，如圖 7 之解析與判讀依據，位於平均環境軸右側者皆屬鎘累積能力較強之品種，而位於左側者相對較低，圖中虛線標示之投影距離近者，表示對各試驗環境皆具穩定性之品種，反之則否。由圖 8 結果顯示，‘TCS10’ (編號 O)、‘TCS17’ (編號 P) 號及 ‘TCSW2’ (編號 U) 位於平均環境軸最右側，代表這 3 品種鎘累積能力最高，且三者皆為秈型稻，Chiao *et al*. (2019) 先前研究已指出，台灣秈稻的糙米鎘濃度普遍高於稉稻，而且又以 ‘TCS17’ 的環境穩定性較高，表示 ‘TCS17’ 是環境穩定性高的高鎘累積品種。再者，平均環境軸最左側之 ‘KH139’ (編號 J)、‘TT33’ (編號 N)、‘TNGW73’ (編號 R)、‘TKW3’ (編號 T) 及 ‘TKW1’ (編號 S) 等，屬於鎘累積能力低之品種，於 Chiao *et al*. (2019) 的研究中顯示 ‘TKW1’ 及 ‘TKW3’ 的糙米鎘濃度低於 0.3 mg kg^{-1}，屬於相對安全的水稻品種；而且 ‘KH139’、‘TT33’、‘TNGW73’ 及 ‘TKW3’ 等穩定性較高，可歸類為環境穩定性高的低鎘累積品種。

結論

本研究提出 2 處試驗區經連續 2 期作水稻生產的糙米鎘濃度皆與根圈土壤鎘濃度成正比，而且秈型稻糙米鎘累積濃度皆有較高之趨勢，依糙米鎘濃度或 BCF 值皆顯示 ‘TCS10’、‘TCS17’、‘TCSW2’ 等秈型品種的鎘累積能力較強，而 ‘TKW1’、‘KH139’、‘TT30’、‘TKW3’、‘TT33’、‘TNGW73’ 等稉型品種較弱。再經 GGE 雙軸圖分析結果，顯示上述糙米鎘累積濃度較低之品種，又以 ‘KH139’、‘TKW3’、‘TNGW73’ 及 ‘TT33’ 是環境穩定性較高的低鎘累積品種；而且導入 GGE 雙軸圖整合分析試驗結果，可進一步評估適合不同栽培環境的

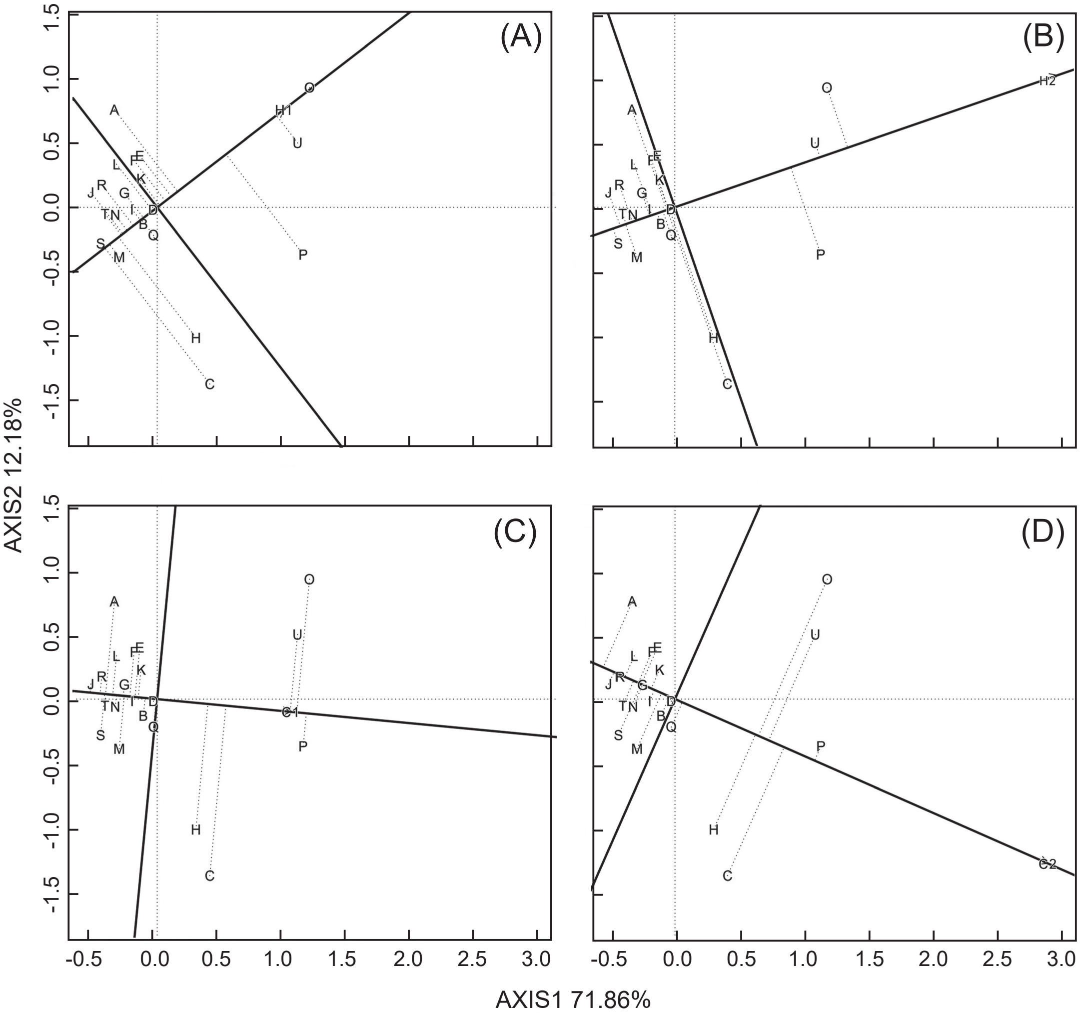


圖 7. 以 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米鎘濃度的 GGE 雙軸圖，解析各品種對應不同試驗環境向量軸 (H1、H2、C1 及 C2) 的投影距離分布：(A) 后里第一期作 (H1)、 (B) 后里第二期作 (H2)、 (C) 彰化第一期作 (C1) 與 (D) 彰化第二期作 (C2)。各水稻品種代號依序如下列：A、B、C、D、E、F、G、H、I、J、K、L、M、N、O、P、Q、R、S、T 及 U。

Fig. 7. Projected lengths on the axes of environment vectors drawn with bold solid lines for the first and second crops in the Houli (HL) site are denoted as (A) H1 and (B) H2 and those in the Changhua (CH) site are denoted as (C) C1 and (D) C2 for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’), which are denoted as the capital letters, A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, and U, respectively in the GGE bi-plot of brown rice Cd concentrations.

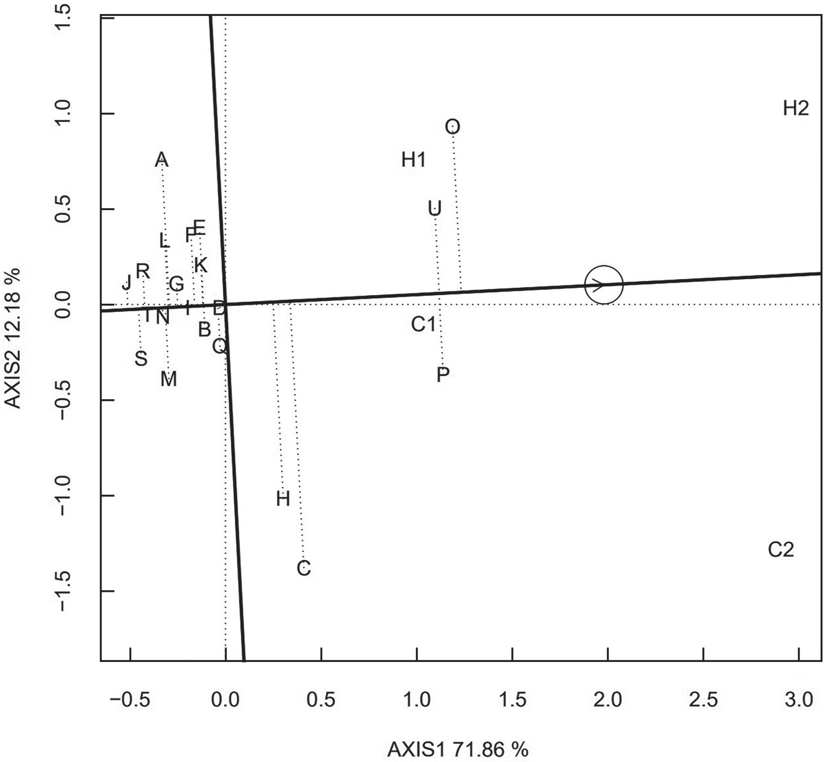


圖 8. 以 21 種水稻 (‘TK2’、‘TK8’、‘TK9’、‘TK14’、‘TK16’、‘TY3’、‘TNG71’、‘TC192’、‘TN11’、‘KH139’、‘KH145’、‘KH147’、‘TT30’、‘TT33’、‘TCS10’、‘TCS17’、‘KHS17’、‘TNGW73’、‘TKW1’、‘TKW3’ 及 ‘TCSW2’) 栽培種糙米鎘濃度的 GGE 雙軸圖，解析各品種對應平均環境軸之投影距離分布。各水稻品種代號依序如下列：A、B、C、D、E、F、G、H、I、J、K、L、M、N、O、P、Q、R、S、T 及 U；后里 (HL) 試驗區第一與第二期作的環境向量終點分別標示為 H1 與 H2；彰化 (CH) 試驗區第一與第二期作的環境向量終點分別標示為 C1 與 C2；小圓圈代表環境向量和的平均環境座標，粗實線為通過座標軸原點以及平均環境座標的平均環境軸，箭頭方向代表糙米鎘累積量較高的環境條件。

Fig. 8. Projected lengths on the average axis of environments drawn with bold solid line for the 21 paddy cultivars (i.e., ‘TK2’, ‘TK8’, ‘TK9’, ‘TK14’, ‘TK16’, ‘TY3’, ‘TNG71’, ‘TC192’, ‘TN11’, ‘KH139’, ‘KH145’, ‘KH147’, ‘TT30’, ‘TT33’, ‘TCS10’, ‘TCS17’, ‘KHS17’, ‘TNGW73’, ‘TKW1’, ‘TKW3’, and ‘TCSW2’), which are denoted as the capital letters, A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P, Q, R, S, T, and U, respectively in the GGE bi-plot of brown rice Cd concentrations. The environment vectors for the first and second crops in the Houli (HL) site are denoted as H1 and H2; the environment vectors for the first and second crops in the Changhua (CH) site are denoted as C1 and C2. The small open circle represents the sum of environment vectors as the average of environments, the bold solid line is the average axis of environments, and the arrow directs the environmental tendency toward Cd accumulation in brown rice.

低鎘累積品種，並調整適合進行篩選試驗的環境條件以提升篩選結果的可信度。彙總本研究結果，對於不同水稻品種吸收鎘與糙米鎘累積濃度之影響因素評估，可見不同試驗區土壤鎘濃度是決定不同生態群的主要因子，而一、二期作氣候條件與管理因素則是次要因子，而且從不同品種基因型的表現，可清楚看到秈稻鎘累積量遠高於稉稻，而稉稻品種間之鎘累積差異則不顯著，可見在管理糙米鎘濃度上，依序從土壤濃度、管理條件、品種推薦逐層著手，最終可穩定降低水稻生產鏈的鎘污染風險。

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Using GGE Bi-Plot to Evaluate Paddy Genotypic Variation in Cd Concentrations of Brown Rice and Interaction with Environmental Effects

Chien-Hui Syu[1], Yu-Wen Lin[1], Bo-Jiun Yang[2], Sung-Lin Chuang[3], Chia-Yi Cheng[2], and Kai-Wei Juang[4,*]

Abstract

Syu, C. H., Y. W. Lin, B. J. Yang, S. L. Chuang, C. Y. Cheng, and K. W. Juang. 2023. Using GGE bi-plot to evaluate paddy genotypic variation in Cd concentrations of brown rice and interaction with environmental effects. J. Taiwan Agric. Res. 72(2):161–179.

Paddy rice (*Oryza sativa* L.) is the staple food of Asian countries. However, paddy rice cadmium (Cd) pollution continuingly happened due to the increase in soil Cd concentration and the issue has attracted more attention. As well known, the accumulation of Cd in rice grains is affected by paddy variety, environmental effects, and the interaction effects between variety and environment. In this study, the GGE bi-plot was used to analyze the main effect of genotype (G) and the interaction effect between genotype and environment (G × E) on Cd accumulation in rice grains. According to the scores of principle components obtained from GGE analysis for different rice varieties, the bi-plots thus were drawn to illustrate the levels of environmental effect on rice genotypes. There were two Cd-contaminated sites in Houli District (HL), Taichung City, and Changhua City (CH), respectively, for a multiple-environment trail to plant 21 rice cultivars in 2 crop seasons. The Cd concentrations in rhizosphere soil samples and brown rice for the rice varieties were measured, and then the observed data were used to screen out the environmentally stably low Cd-accumulation varieties. The results show that most indica varieties had higher cadmium accumulations than japonica rice did. The profiles of brown rice Cd concentrations for the paddy varieties in sites HL and CH are different. The lower Cd-accumulation varieties in HL were not completely consistent with those in CH. This revealed that the interaction between genotype and environment would be a dominant figure in brown rice Cd concentrations. In addition, according to the GGE analysis, the effect G and the interaction effect G × E on Cd accumulation in rice grains have been transported into the 2 principal components (i.e., PC1 and PC2), which were mutually independent and dominated the variation of G and G × E approaching to 84%. The bi-plot gave PC1 and PC2 as the coordinate axes were used to quantify the variation due to environmental effects in brown rice Cd concentration for each paddy variety. The brown rice Cd concentrations of the varieties, ‘KH139’, ‘TKW3’, ‘TNGW73’, and ‘TT33’, were relatively low and less varied with different sites and cropping seasons. That is, they would be the more environmentally stably low Cd-accumulation varieties. Therefore, Cd concentration and availability in the soil will dominate the Cd absorption by different paddy rice varieties and accumulation in brown rice; the weather condition and management practices in different cropping periods may be a secondary factor. In a comparison of indica and japonica types for accumulation of Cd in brown rice, the indica was superior to the japonica. The relatively stable Cd low-absorption varieties all were japonica rice. One can try to reduce the risk of brown rice Cd being higher than the threshold (i.e., 0.4 mg kg^{-1}) by selecting environmentally stably low Cd-accumulation varieties and adopting the agronomic practices to go into a decline of soil Cd availability.

Key words: Cadmium, Paddy rice, GGE bi-plot, Interaction between genotype and environment, Cultivar.

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